An-Najah National University

Faculty of Graduate Studies

Resistance of Staphylococcal and Streptococcal Clinical Isolates to Macrolides and Functionally Related Antibiotics in Nablus District

By

Naela Khaled Asad Sabbah

Supervisor

Dr. Motasem Al-Masri

Co- Supervisor

Dr. Nael Abu Hasan

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This thesis was defended successfully on 1/6/ 2014 and approved by:

Defense Committee Members	Signature
- Dr. Motasem Y. Al-Masri \Supervisor	.....
- Dr. Nael Abu Hasan S. \Co-Supervisor	.....
- Dr. Mohammad A. Farraj \External Examiner	.....
- Dr. Kamel M. Adwan \Internal Examiner	.....

Dedication

To My Family and Friends with Respect and Love

Acknowledgments

I would like to express my deepest sense of gratitude to my supervisors Dr. Motasem Al-Masri and Dr. Nael Abu-Hasan for their patient guidance and encouragement and for reading and approving the thesis.

Thanks for faculty members of Graduate Studies at An-Najah national University for their support during my master program.

Finally, special thanks are extended to my dear husband for his support. Similar thanks are extended to my beloved parents, daughter, brothers, sisters and relatives.

الإقرار

أنا الموقعة ادناه مقدم الرسالة التي تحمل العنوان:

Resistance of Staphylococcal and Streptococcal Clinical Isolates to Macrolides and Functionally Related Antibiotics in Nablus District

دراسة حول مقاومة بعض الأنواع البكتيرية للمضادات الحيوية من النوع (Macrolides) والمشابهة لها وظيفيا في منطقة نابلس

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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Resistance of Staphylococcal and Streptococcal Clinical Isolates to Macrolides and Functionally Related Antibiotics In Nablus District

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Dr. Motasem Al-Masri
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Abstract

A total of 200 Staphylococcal and 52 Streptococcal clinical bacterial isolates were collected from January 2012 to April 2013 from different clinical centers in Nablus district. Minimal inhibitory concentration (MIC) values of erythromycin and clindmycin were determined using agar dilution method. Micro-broth dilution method was only applied for *S. pneumoniae* isolates. A representative 47 isolates of erythromycin resistant strains were examined for antibiotic resistance genes (*ermA*, *ermB*, *ermC*, *msr*, *mef*, and *ere*) by PCR. MIC values of erythromycin and clindamycin, erythromycin-clindamycin induction test and data on resistant genes were combined to predict the most probable mode of resistance among the studied isolates. Relatively high frequencies of erythromycin resistance were found among Streptococci (63.5%) and Staphylococci (65.5%) isolates. The frequency of erythromycin resistance among coagulase negative Staphylococci (CONS) was 76.9%, which was higher than that among *S. aureus* (64.7%). With respect to clindamycin resistance, 48.1% of Streptococci and 20.5% of Staphylococci isolates were resistant. Resistance of Staphylococci isolates to erythromycin appears to be mediated by efflux mechanism (MS phenotype, 50.4%) and target site

modification (MLS_B phenotypes, 49.6%). Expression of MLS_B phenotype in staphylococci was constitutive in 61.5% and inducible in 38.5% of the isolates. Among Streptococci isolates, resistance to erythromycin was most commonly (75.8%) mediated by target modification (MLS_B). However, efflux mechanism of resistance (M phenotype) was detected in 24.2% of the isolates. Among the 36 Staphylococcal isolates analyzed by PCR, *msr* gene was detected in 20 (55.6%), *ermC* in11 (30.6%) and *ermA* in 9 (25%). On the other hand, among examined Streptococcal isolates (11), *ermB* gene was detected in 9 (81.8%) of isolates, *mef* in 3 (27.3%), *ere* in 1 (9.1%) and *ermC* in 1 (9.1%).

The percentage of erythromycin resistant Staphylococci was highest among infants 0-2 years old (74.5%) and older age group >65 years (75%). Similarly, clindamycin resistance among Staphylococci was highest in bacteria isolated from patients >65 years (50%). This was significantly higher than that among 3-14 year age group (3.5%, ***P***= 0). Staphylococci isolates recovered from gynecology department showed the highest erythromycin resistance when compared to isolates from other departments and variations in resistance rates were significant (***P***=0.000). Erythromycin resistance among Staphylococci bacteria isolated from blood and nasal swab were significantly higher than that among wound swabs (***P***=0.000).

Chapter One
Introduction

1.1 Introduction

Results of national and global surveillance studies indicate that the incidence of isolation of antimicrobial-resistant pathogens in healthcare institutions is increasing and becoming common (1, 2, 3, 4, 5, 6, 7, 8, 9). Bacterial resistance often results in treatment failure, which can have serious consequences, especially in critically ill patients. Ineffective empiric antibiotic therapy, has resulted in increased mortality rates in patients with bloodstream infections caused by resistant *Pseudomonas aeruginosa*, Staphylococci spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., and enterococci (10, 11, 12, 13, 14). The challenge of isolation of resistant bacteria is not only within healthcare institutions but may also spread in communities as well (5, 15).

Surveillance studies carried out on antimicrobial agents were essential for establishing trends in antimicrobial resistance of pathogens and for recognition of emerging pathogens at different levels, i.e., national and global. Such studies helped in the development of targeted approaches to control antimicrobial resistance (16).

1.2 General characteristics of Staphylococci

The genus Staphylococcus is composed of several species, many of which may be encountered in human clinical specimens (17). Staphylococci are spherical cells arranged in irregular clusters similar to grape appearance. However, single cocci, pairs, tetrads, and chains are also seen (18, 17). Staphylococci bacteria are nonmotile, non-spore-forming, catalase-positive and gram-positive cocci (18, 17). The organisms are generally found on the skin and mucous membranes of humans. In humans, some of these pathogens produce the enzyme coagulase, which is used for laboratory identification for these organisms (17, 18).

1.3 Clinical significance of Staphylococci

Among the genus staphylococcus, the three main species of clinical importance are *S. aureus, S. epidermidis, and S. saprophyticus* (18). *S. aureus* is coagulase positive and is a major pathogen for human (18, 17). It is found in the external environment and the anterior nares of 20-40% of healthy adults. Other sites of colonization include intertriginous skin folds, the perineum, and the vagina. Although this organism is frequently a part of the normal human microflora, it can cause significant opportunistic infections under the appropriate conditions (17). *S. aureus* is the most virulent encountered Staphylococcus species. It produces exo-toxins causing diseases such as toxic shock and Staphylococcal scalded skin syndromes. In addition, *S. aureus* can make direct invasion and

systemic dissemination resulting in diseases like bacteremia, septic shock syndrome, skin infection and abscesses (19, 20).

Coagulase-negative Staphylococci (CONS) are increasing in importance as cause of hospital-acquired infections, particularly nosocomial bacteremias (21), and neonatal sepsis (22). The National Nosocomial Infection Survey (NNIS) reported that the incidence of CONS as a cause of nosocomial bacteremias increased from 9 to 27% during the period 1980 to 1989, to become the most common single cause of these infections (21). It was reported that there is an association between the dramatic increase in CONS as a cause of nosocomial bacteremias and the increase rate of resistance of these pathogens to antimicrobial agents (23).

Among coagulase negative staphhylococci, *S. epidermidus* and *S. saprophyticus* are frequently reported in human infections (17). *S. epidermidis* is widely recognized as one of the etiologic agents of bacteremia, postoperative cardiac infections endocarditis, osteomyelitis, urinary tract infections with a frequent association with colonization of intravascular catheters and orthopedic devices (12, 14). *S. saprophyticus* is known to causes urinary tract infections in young women (18).

1.4 General characteristics of Streptococci

Streptococci bacteria represent a large group of gram-positive microorganisms of remarkable heterogeneity. Most Streptococci are

facultative anaerobes, nonmotile, and tend to grow in chains of variable length, especially during cultivation in vitro (24, 18). Some Streptococci produce a capsular polysaccharide (18).

1.5 Clinical significance of Streptococci

Streptococcus pneumoniae (*S. pneumoniae*) is an etiological agent of pneumonia. It's a major killer of humans, albeit its lethality frequently arises as a complication of a preceding debilitating illness (24). *S. pneumoniae* may also cause complications such as meningitis, endocarditis and septic arthritis (18). *Streptococcus pyogenes* is the most frequent bacterial cause of pharyngitis; this bacterium also causes impetigo, rheumatic fever and glomerulonephritis (25, 18). Viridans Streptococci are identified to cause systemic diseases such as bacteraemia, bacterial endocarditis, especially in patients with decreased white blood cells counts or patients with pneumonia (26, 27).

Streptococcus agalactiae is a pathogen of growing importance in human pathology. It is the most important cause of neonatal sepsis (28, 29) and meningitis in newborn infants (30, 31).

1.6 Macrolides, Lincosamides and Streptogramin B (MLS_B) Antibiotics

MLS_B antibiotics are chemically distinct, but have similar mode of action against bacterial cells (32, 33, 34, 35), therefore, common characteristics will be discussed together.

1.6.1 Structures of antibiotics

1.6.1.1 Macrolides

Macrolides antibiotics consist of a macrocyclic lactone ring containing 14, 15 or 16 atoms with neutral or amino sugars linked via glycosidic bonds (Figure 1) (36, 37).

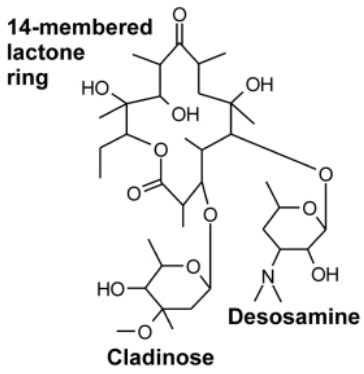

Figure 1.1 Chemical structure of macrolides (erythromycin) (36).

According to the number of atoms in the lactone nucleus, macrolide antibiotics can be categorized into three groups. Macrolides possessing 14-membered lactone ring includes erythromycins, oleandomycin, roxithromycin, dirithromycin, clarithromycin and flurithromycin, whereas 15-membered antibiotics include azithromycin. Examples of 16-membered macrolides include josamycin, rosaramicin, rokitamycin, kitasamycin, mirosamycin, spiramycin and tylosin. Both of spiramycin and tylosin antibiotics are used almost exclusively in treatment of animals (38).

1.6.1.2 Lincosamide

Lincosamide class of antibacterial agents originates from a natural product, lincomycin (Figure 2) and includes semisynthetic derivatives, clindamycin and pirlimycin (39). Lincomycin is composed of an amino acid (propylhygric acid) (40), linked via a peptide bond to a sugar moiety (methylthiolincosamide) (41, 42).

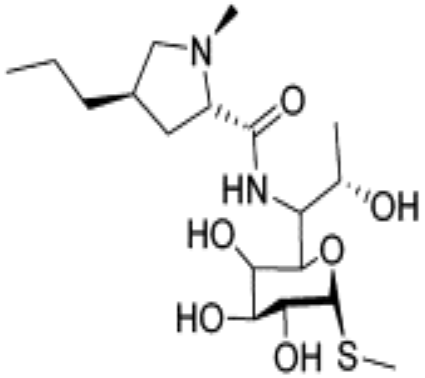

Figure 1.2 Chemical structure of lincomycin (40, 41, 42).

1.6.1.3 Streptogramin B

The streptogramin family is subdivided into A and B groups or alternatively into M and S groups, respectively. Streptogramin B consists of several modified amino acids as shown in Figure 1.3 (43, 44).

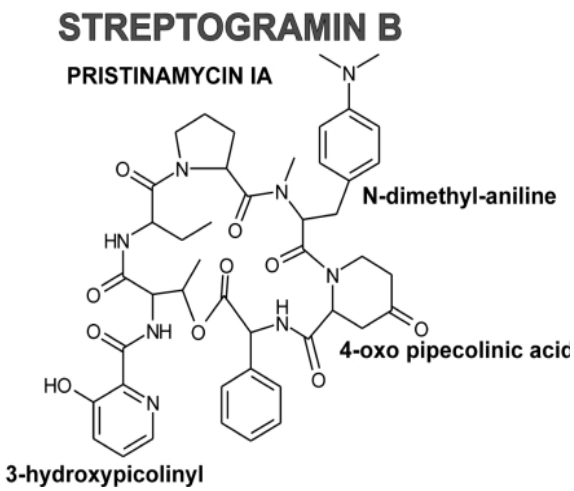

Figure 1.3 Chemical structure of a Streptogramin B (43).

1.6.2 Mechanism of action and applications of MLS_B Antibiotics

All MLS_B antibiotics inhibit protein synthesis. MLS_B antibiotics bind to the large 50S ribosomal subunit, close to the peptidyl transferase center (45, 46, 47, 48, 49, 50, 51, 52). This binding was reported to block peptide bond formation and/or peptidyl-tRNA translocation from the A to the P site of the ribosome (50, 51, 52). This center is composed entirely of RNA (53, 54, 55, 56). Several alterations in 23 S ribosomal RNA, give resistance against all members of the MLS_B group (57).

MLS_B antibiotics are widely used in the treatment of Staphylococcal and Streptococcal infections (58, 59). MLS_B drugs are recommended as alternative treatment of patients, who are allergic to *B*-lactam antibiotics (60, 61, 58). Moreover, erythromycin and other macrolides are considered alternative treatment for Streptococcal pharyngitis and other non-serious infections caused by *S. pyogenes* (62,

63, 64). MLS_B drugs are recommended for penicillin-resistant Viridians Streptococci (60, 61, 58). Macrolides has been used as therapy in severe cases of acne. It is also the agent of choice in treating whooping cough, Campylobacter and Mycoplasma infections, and legionnaires disease (65). Although, lincosamide are mostly active against gram-positive organisms it's also used against selected gram-negative anaerobes and protozoa (66, 67).

1.6.3 Resistance mechanisms to MLS_B antibiotics

Although macrolide, lincosamide and streptogramin B antibiotics possess different chemical structures, they are functionally overlapping. Thus, a discussion of the mode of resistance to macrolide antibiotics must include lincosamide and streptogramin B families (44). The expanded therapeutic application of macrolide, lincosamide and streptogramin antibiotics to different infection types has been associated with increased numbers of resistant strains among Staphylococci and Streptococci (32, 33, 34, 35**,** 68). Bacterial resistance to MLS antibiotics may be expressed through different mechanisms including target site modification, efflux pump and enzymatic inactivation of antibiotic (69, 70, 71, 57, 72, 73, 74).

1.6.3.1 Target modification

Target modification occurs at the level of the ribosomes via a 23S rRNA methylase enzyme. This enzyme is encoded by erythromycin resistance methylase (*erm*) gene (69, 71, 57). There are several classes of

erm genes distinguishable by hybridization criteria (69, 71, 57). Examples of *erms* include *ermA*, *ermB*, and *ermC* (75, 69, 35), *ermF* (76), *ermY* (77). Methylases enzyme adds one or two methyl residues to a highly conserved adenine residue in domain V (the peptidyl transferase center) of 23S rRNA (78, 57, 69). This ribosomal modification makes the bacterial strain resistant to most macrolides, lincosamides, and streptogramin B compounds; phenotypically, this resistance pattern is known as MLS_B resistance (79, 78, 69). Expression of MLS_B resistance can be inducible or constitutive and is unrelated to the class of an *erm* determinant (80, 81, 69, 82, 83). In Staphylococci and Streptococci bacteria, constitutive expression of MLS_B resistance can lead to cross-resistance to macrolides, lincosamides and streptogramin B ($cMLS_B$) (resistance includes 16-membered ring macrolides) (84, 72). In Staphylococcal bacteria, inducible MLS_B resistance strains possess resistance to 14- and 15-membered ring macrolides and susceptibility to 16-membered ring macrolides (84). Staphylococcal isolates with inducible resistance phenotype show in vitro resistance to erythromycin and susceptibility to clindamycin (85, 86, 87, 88). Such bacterial strains possess *erm* genes, which require an inducing agent to express resistance to clindamycin. For example erythromycin antibiotic can act as a strong inducer of methylase enzyme production (89). Clindamycin therapy of infections caused by bacteria with inducible resistance phenotype can lead to development of clindamycin resistance and consequently clinical failure (85, 86, 87, 88).

In Streptococcal isolates, a variety of phenotypes are produced by expression of inducible resistance. Phenotypes include high or low level of erythromycin resistance, with susceptibility or resistance to clindamycin (72, 90).

1.6.3.2 Efflux mechanism

Staphylococci appear to have an efflux system (91, 92, 70, 93, 94, 95, 96, 84, 97, 98) which is specific for 14- and 15-membered macrolides and streptogramin B antibiotics. Lincosamide antibiotics are not pumped by this staphylococcal efflux system. The resulting resistance pattern is called MS phenotype. The efflux system appears to be multi-component (84, 97). The gene *msrA* (84, 97) encodes ATP-binding proteins that are involved in transport (99, 100, 101). It is clear that *msrA* must be present to confer the macrolide and streptogramin B resistance i.e. MS phenotype (96). In Streptococci, active efflux pump is encoded by *mef* (macrolide efflux) gene (102, 103, 104). The *mef(A/E)* gene causes resistance to 14- and 15-membered macrolides compounds only, and the encoding phenotype is designed M (74). Two subclasses of the *mef* gene have been described, *mef(A)* gene (102), originally found in *S. pyogenes*, and *mef(E)* gene originally found in *S. pneumoniae* (103). The subclass *mef(A)* and *mef(E)* are 90% identical at the nucleotide level but they are endowed with important genetic differences (105).

1.6.3.3 Enzymatic inactivation

Resistance caused by bacterial production of enzymes that inactivate MLS_B antibiotics has been described for a number of clinically important organisms such as *S. aureus* (106, 107, 108, 109, 110, 98) *S. haemolyticus* (106, 108), and *Escherichia coli* (111, 112, 113, 114, 115, 116, 117, 118, 119). Lactone ring of the macrocyclic nucleus can be hydrolysed by certain enzymes such as *EreA* and *EreB*. In addition, macrolides can be inactivated by phosphotransferases, which were reported in *S. aureus* (98). However, enzymatic inactivation in gram-positive bacteria is rarely reported (120).

1.7 Aims of the study

Little information is known about the prevalence of the resistance to macrolides and functionally related antibiotics among Staphylococci and Streptococci clinical isolates in the Palestinian territories, thus, the current study aims at:

1. Determine the prevalence of resistance to macrolides and lincosamides among Staphylococci and Streptococci clinical isolates in northern Palestine, mainly in Nablus district.

2. Determine the phenotypes of resistance to macrolides using Minimum Inhibitory Concentrations (MIC) values of erythromycin and clindamycin, and from induction tests (erythromycin-clindamycin).

3. Detect the molecular mechanism of resistance to macrolide by polymerase chain reaction (PCR) using representative isolates.

Chapter Two
Materials and Methods

2.1 Collection of bacterial isolates

Bacterial isolates were collected from January 2012 to April 2013 from different clinical centers in Nablus district. These centers included Rafedia, Nablus, Al-Arabi and Al-Watani hospitals and New Technology and Medicare medical laboratories (isolate collection from Medicare started at February 2013). Patient demographic data were obtained from laboratory records for each isolate. The information included: name, age, sex, specimen type, clinical center, hospital wards, date of hospital admission and date of specimen collection. Each isolate was given an identity number and stored in 20% glycerol Nutrient Broth at -70 ˚C. Hospital associated infection was defined as occurrence of infection 48 hours or more after hospital admission.

2.2. Identification of bacterial isolates

Identification of bacterial isolates was confirmed by several biochemical tests as mentioned previously by Win et al and Forbes et al (121, 17). Gram stain and catalase tests were performed for all isolates. Identification of Staphylococcal bacteria was based on coagulase test, mannitol salt agar test, aerobical production of acid from maltose, and susceptibility to bacitracin, novobiocin, and polymyxin B. Identification schemes used for Streptococcal isolates included: growth on Blood Agar in absence of pyridoxal (vitamin B6); haemolysis type; growth at 6.5% NaCl supplemented blood agar; susceptibility to bacitracin, trimethoprim-sulfamethoxazole (SXT), optochin and vancomycin; growth at 10^{o}C, and

chromogenic media Uriselect (Bio-Rad, France). All antibiotics were obtained from Oxoid (UK).

2.3 Determination of minimal inhibitory concentration (MIC)

MIC values of erythromycin and clindamycin were determined by agar dilution method for Staphylococcal and Streptococcal strains, while micro-broth dilution method was used for *S. pneumoniae* isolates (Figure 2.1). The applied procedures were according to Clinical Laboratory Standards Institute (CLSI) (121, 122). *S. aureus* ATCC 25923 was included in each run as control strain with susceptibility to both erythromycin and clindamycin antibiotics.

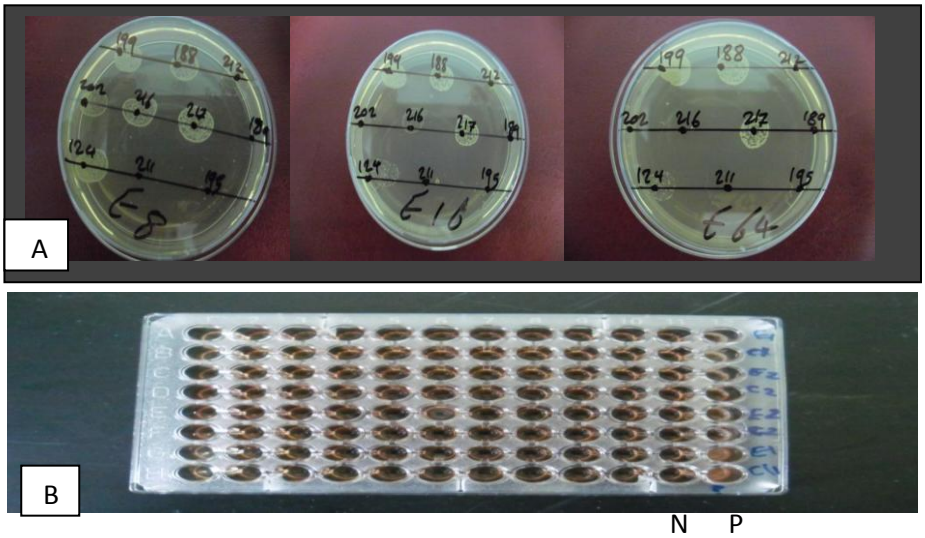

Figure 2.1 Determination of MIC. **A**. Agar dilution method plates. **B**. Micro-broth dilution method.
N, negative growth control; P, positive growth control.

2.3.1 Media preparation

In agar dilution method, flasks containing 50ml (or 47.5ml for Streptococci) of Mueller-Hinton (MH) agar (hy-labs, Israel) were sterilized and placed in water bath at 40 ˚C. To each flask a specific volume of antibiotic solution and for Streptococci 2.5ml blood was/were added, followed by well mixing and pouring into Petridishes. This resulted in MH agar plates with 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128µg/ml concentrations of antibiotics. Plates without antibiotics were prepared to serve as positive control of bacterial growth.

In micro-broth dilution method, 100 μl of MH broth containing 5% lysed sheep blood (lysed by 5 freeze-thaw cycles and distilled water) was dispensed in each well of the microtitre tray. This was followed by the addition of 100 μl of 32µg/ml antibiotic in the first well. After mixing, a 100 µl of sample mixture was transferred to the next well and the process was repeated untill well number 11. A 100µl sample was removed from this well after mixing. The last well (number 12) was antibiotic free and served as positive control of bacterial growth. This resulted in wells containing 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 µg/ml concentrations of antibiotics.

2.3.2 Preparation of bacterial inoculum

Four to five colonies of bacteria from fresh culture were placed in trypticase soy broth (TSB). The turbidity of broth was adjusted to be

equivalent to 0.5 Mcfarland standard ($1.5x10^8$ CFU/ml). In agar dilution method, the bacterial suspension was diluted 1:15 with TSB to achieve a concentration of $1x10^7$CFU/ml. For micro-broth dilution method, the bacterial suspension was diluted 1:3 with TSB to achieve a concentration of $5x10^7$CFU/ml.

2.3.3 Inoculation of bacterial isolates

A 1µl of bacterial suspension (10^4 CFU/spot) was transferred to the MH agar plates containing different concentrations of antibiotics. Inoculum's spots were allowed to dry at room temperature before inverting the plates and the plates were then incubated at 35˚C for 18 hours.

In micro-broth dilution method, 1µl of bacterial suspension, possessing a concentration of $5x10^7$CFU/ml, was transferred to each well except well number 11, which was used as a negative control of bacterial growth. Microtitre tray was covered and incubated at 35˚C for 18 hours.

2.3.4 Interpretation of results

The MIC was considered to be the lowest concentration of the agent that completely inhibits visible growth as judged by the naked eye (121). MIC break points of erythromycin and clindamycin were based on CLSI (122). Staphylococcal bacterial isolates were considered susceptible to erythromycin when MIC was $\leq$ 0.5µg/ml, intermediate 1-4 µg/ml, and resistant when MIC $\geq$ 8 µg/ml. Clindamycin break points for

Staphylococci isolates were as follows: susceptible, ≤ 0.5 µg/ml; resistant, ≥ 4 µg/ml; and intermediate 1-2 µg/ml.

A Streptococci bacterial isolate (including *S. pneumoniae*) was considered susceptible to erythromycin or clindamycin when MIC was ≤0.25 µg/ml and was considered resistant when MIC was ≥1 µg/ml. An isolate with erythromycin MIC value of 0.5 was considered an intermediate resistant isolate.

2.4 Detection of inducible MLS_B phenotype

This test was performed for isolates that were resistant to erythromycin but susceptible to clindamycin. For detection of inducible MLS_B phenotype, double disk diffusion method (D-test) was performed according to CLSI guidelines (123). A 24 hour old bacterial culture was used to prepare a suspension in normal saline equivalent to 0.5 McFarland. The Staphylococcal bacterial suspension was then inoculated onto a Mueller - Hinton agar (MH) plate, while Streptococcal suspension was inoculated onto MH supplemented with 5% blood. Erythromycin (15µg) disk was placed 15 mm (edge to edge) apart from clindamycin (2µg) disk on inoculated MH Plates. The plates were incubated for 18 hours at 35°C. Isolates with D-shape zone around the clindamycin, were interpreted as positive for inducible resistance (D-test positive) as shown in Figure 2.2.

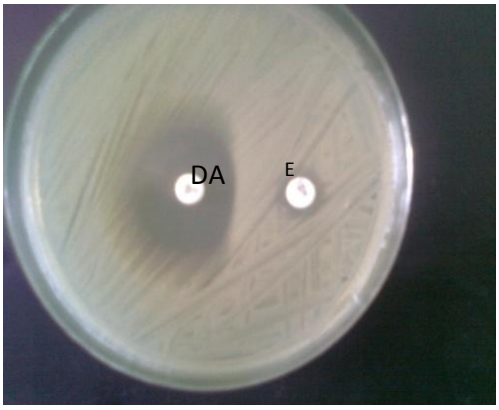

Figure 2.2 Inducible MLS_B phenotype. E, erythromycin; DA, clindamycin;

2.5 Detection of methicillin resistance

This test was performed for Staphylococci isolates. Resistance to oxacillin antibiotic was detected by disk diffusion test. Preparation of bacterial suspension and plates was similar to that previously described for inducible MLS_B phenotype; however, the media was supplemented with 5% NaCl when *S. aureus* isolates were examined. Oxacillin disk (1μg) was applied to inoculated media and the plates were then incubated at 30°C for 18 hours. A zone of inhibition ≤ 10 mm and ≤ 17 mm indicated *S. aureus* and CONS resistance to oxacillin, respectively (122).

2.6 Detection of Resistant Genes

2.6.1 DNA extraction

Bacterial isolates were grown on nutrient or blood agar for 24 hours. Colonies (2-3) were transferred to Eppendorf tube containing 600μl of Tris acetate - EDTA (TAE) buffer and mixed well to form a suspension. After one hour, Eppendorf tubes were centrifuged at 4000g

for 5 minutes and supernatant was discarded. A volume of 600µl distilled water was added to the pellet and tubes were placed in boiling water for 15 minutes and left to cool down at room temperature. An equal volume of chloroform was added and mixed followed by brief centrifugation. The upper layer containing DNA material was aspirated and placed in a new Eppendorf tube. Chloroform extraction step was repeated to ensure that DNA is protein free sample. Extracted DNA was stored at - 20˚C.

2.6.2 Polymerase chain reaction (PCR)

The reagents of PCR were obtained from SIGMA-ALDRICH (USA). The final reaction mixture (25µl) contained 1x PCR solution [1.5 units Taq DNA polymerase, 10mM Tris-HCl, 50 mM KCl, 1.5 $MgCl_2$, 0.001% gelatin, 0.2mM deoxynucleoside triphosphate (dNTP)]. A concentration of 0.8pmole/µl was used for each of the used primers. With respect to $MgCl_2$ concentration it was 2mM for *ermA*, *ermB*, *ermC*, *msr* primers (4mM $MgCl_2$ concentration was used when each primer pair was applied alone) or 4mM $MgCl_2$ for the *ere* and *mef* primers (124).

The PCR assays were made using **T**professional standard Thermocycler (Biometra GmbH, Germany). PCR mixtures were subjected to thermal cycling as follows: 5 minutes at 94˚C and then 40 cycles of 1 minute at 94˚C for the denaturation step and 1.5 minutes at 45˚C for the annealing step and 2 minutes at 72˚C for the extension step. This was followed by a final extension step at 72˚C for 7 minutes. PCR products were detected by 1.5% agarose gel electrophoresis with ethidium

bromide staining. Primers, their corresponding sequences, expected size of PCR products, and their reference article are listed in Table 2.1.

2.7 Statistical Analysis

Minitab version 15.0 was used by a statistical specialist. Chi-square and Fisher's exact tests were applied for comparison of the resistance frequencies among different groups and prevalence of resistance to antibiotics in different age groups. Independent *t*-test was applied for comparison of the mean values among different age groups. A ***P***-value <0.01 was considered statistically significant.

Table 2.1 Primers used in the study.

Primer	Primer sequence	Expected size of PCR product (b.p*)	Reference
ermC1:	5'GCTAATATTGTTTAAATCGTCAATTCC –3'		(125)
ermC2:	5' GGATCAGGAAAAGGACATTTTAC –3'	572	(125)
ermB1	5'-GAAAAGGTACTCAACCAAATA-3'		(124)
ermB2	5'- AGTAACGGTACTTAAATTGTTTAC-3'	639	(124)
ermA1	5'-TCTAAAAAGCATGTAAAAGAA-3'		(124)
ermA2	5'-CTTCGATAGTTTATTAATATTAGT-3'	645	(124)
msrA1	5'- GGCACAATAAGAGTGTTTAAAGG-3		(84)
msrA2 :	5'- AAGTTATATCATGAATAGATTGTCCTGTT-3'	399	(84)
mefA/E1	5'-AGTATCATTAATCACTAGTGC-3		(124)
mefA/E2	5'-TTCTTCTGGTACTAAAAGTGG-3	348	(124)
ereA1	5'-AACACCCTGAACCCAAGGGACG-3'		(119)
ereA2	5'-CTTCACATCCGGATTCGCTCGA-3'	420	(119)

*base pair

Chapter Three

Results

3. 1 Bacterial isolates

A total of 252 bacterial isolates were collected from January 2012 to April 2013. Isolates were collected from different types of clinical specimens and one positive culture per patient was included. Bacterial isolates included 200 Staphylococci and 52 Streptococci isolates. Isolates were obtained from the following hospitals and private laboratories in the city of Nablus: Rafidia hospital (160), New Technology laboratory (35), Nablus Specialty Hospital (26), Al-Arabi Specialty Hospital (19), Al-Watani Hospital (7) and Medicare laboratory (5) as shown in Table 3.1. Staphylococci isolates comprised of 187 *S. aureus* and 13 coagulase negative Staphylococci (12 *S. epidermidis* and 1 *S. saprophyticous* isolates). Streptococci isolates included 33 *Streptococcus agalactiae*, 14 Viridans Streptococci, 3 *S. pneumoniae*, and 2 *S. pyogenes*.

Staphylococci isolates were recovered from various clinical materials of 56 outpatients and 144 patients hospitalized in 10 different departments. Among the hospitalized patients, the frequency of Staphylococcal bacterial isolation was highest in general surgery unit (32 isolates) and ranged from 6 to 17 isolates in the rest of units. The majority of Streptococci isolates were obtained from outpatients (Table 3.1).

Table 3.1 Source of Staphylococci and Streptococci isolates.

Variable	Total number	Staphylococci isolates No.	Streptococci isolates No.
Source			
Rafedia hospital	160	140	20
New Technology laboratory	35	9	26
Nablus Specialty hospital	26	21	5
Al-Arabi Specialty hospital	19	18	1
Al-Watani hospital	7	7	0
Medicare-laboratory	5	5	0
Units			
Outpateints	92	56	36
Inpatients	160	144	16
General surgery	37	32	5
Emergency	20	17	3
Pediatrics	18	16	2
Burns	16	16	0
Neonates	15	14	1
Urology	14	14	0
ICU*	11	9	2
Internal medicine	15	13	2
Orthopedic	7	7	0
Gynecology	7	6	1
Specimen			
Wound swab	143	126	17
Urine	42	23	19
Blood	10	8	2
Sputum	9	6	3
Nasal swab	8	8	0
Fluid	6	5	1
Throat swab	6	1	5
Vaginal swab	5	2	3
Umbilical swab	5	5	0
Ear swab	3	2	1
Semen	3	3	0
Skin	2	2	0
Burn swab	2	2	0
Tissue	2	2	0
C.V.P *	1	1	0
Chest swab	1	1	0
CSF*	1	0	1
Pus	1	1	0
Drain	1	1	0
Breast discharge	1	1	0
Sex			
Male	132	113	19
Female	120	87	33
Total	252	200	52

* ICU, Intensive care unit; CVP, central venous catheter; CSF, Cerebrospinal fluid

Most of the Staphylococci isolates were recovered from wound swab (126 isolates) followed by urine (23), blood (8), nasal (8) and sputum (6) specimens (Table 3.1). Staphylococcal infections were more common among males (56.5%). Streptococci isolates were recovered predominantly from urine (19 isolates) and wound swabs (17) as shown in Table 3.1. The frequency of Streptococcal infection is higher among females (27.5%) compared to males (14.4%).

In the present study, patients were grouped into 6 age groups. Distribution of bacterial isolates among various age groups was as follows: 0-2 year (51 Staphylococci and 4 Streptococci), 3-14 (29 Staphylococci and 3 Streptococci), 15-39 (36 Staphylococci and 20 Streptococci), 40-65 (31 Staphylococci and 10 Streptococci), > 65 (12 Staphylococci and 2 Streptococci) and unknown age (41 Staphylococci and 13 Streptococci). Staphylococci and Streptococci isolates were recovered during all months of the year.

3.2 Susceptibility of Staphylococci and Streptococci isolates to erythromycin and clindamycin

Table 3.2 shows the percentage of resistance to erythromycin and clindamycin among the bacterial isolates. A total of 131 (65.5%) Staphylococci isolates were resistant to erythromycin. A much lower frequency of resistance to clindamycin (20.5%) was found among Staphylococci isolates. Data presented in Figure 3.1 shows resistance percentages of major bacterial groups. The MIC for erythromycin resistant

Staphylococci isolates ranged from 8 to ≥ 128µg/ml. MIC of clindamycin resistant isolates ranged from 4 to ≥128µg/ml. The frequency of erythromycin resistance among coagulase negative Staphylococci (CONS) was 76.9%, which was higher than that among *S. aureus* (64.7%). Differences between CONS and *S. aureus* were not significant (***P***= 0.317). In contrary, higher clindamycin resistance rate was detected among *S. aureus* (20.9%) strains without significant deference (***P***= 0.600) as shown in Table 3.2 and Figure 3.1.

Table 3.2 Resistance of erythromycin and clindamycin in different bacterial species included in the study.

Bacterial species	No	Erythromycin			Clindamycin		
		Resistant No* (%)	Intermediate No* (%)	Susceptible No* (%)	Resistant No (%)	Intermediate No (%)	Susceptible No (%)
Staphylococci	200	131(65.5)	6(3)	63(31.5)	41 (20.5)	6(3)	153 (76.5)
S. auerus	187	121(64.7)	6(3.2)	60(32.1)	39(20.9)	5(2.7)	143(76.5)
CONS*	13	10(76.9)	0(0)	3(23.1)	2(15.4)	1(7.7)	10(76.9)
S.epidermidis	12	10(83.3)	0(0)	2(16.7)	2(16.7)	1(8.3)	9(75)
S.saprophyticus	1	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)
Streptococci	52	33(63.5)	0(0)	19(36.5)	25(48.1)	0(0)	27(51.9)
S. agalactiae	33	26(78.8)	0(0)	7(21.2)	20(60.6)	0(0)	13(39.4)
Viridans *S.*	14	5(35.7)	0(0)	9(64.3)	3(21.4)	0(0)	11(78.6)
S.pneumoniae	3	0(0)	0(0)	3(100)	0(0)	0(0)	3(100)
S.pyogenes	2	2(100)	0(0)	0(0)	2(100)	0(0)	0(0)

* No, number; CONS, coagulase negative Staphylococci.

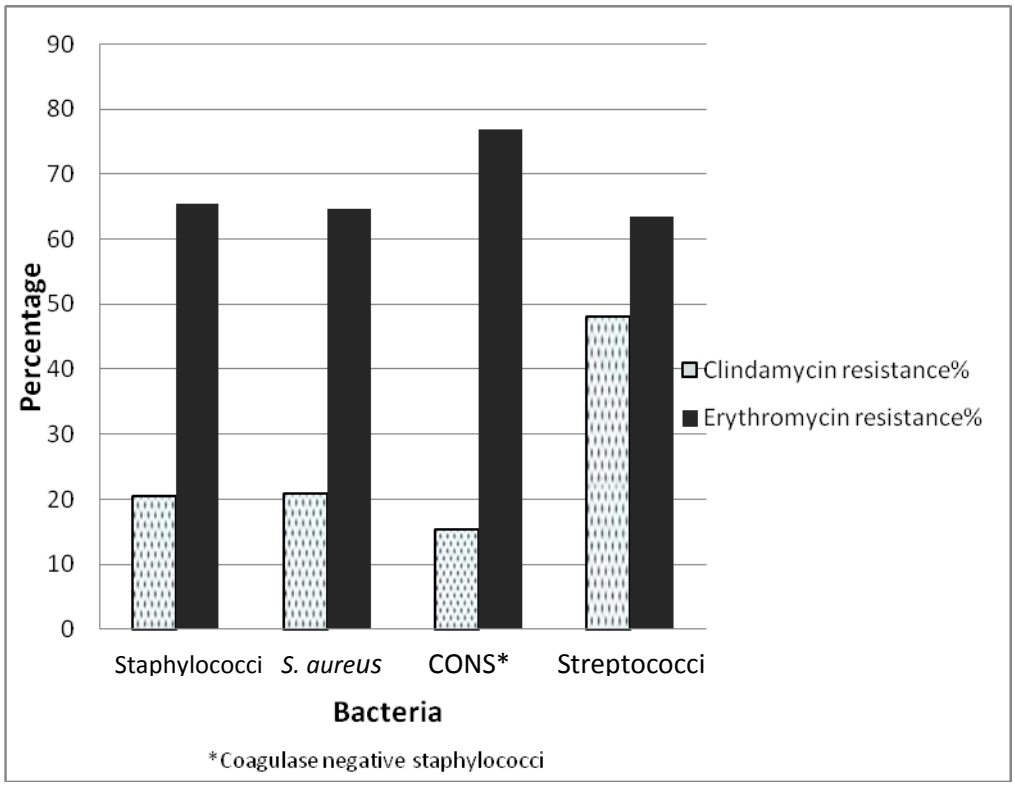

Figure 3.1 Percentages of erythromycin and clindamycin resistance among Staphylococci and Streptococci isolates.

The frequency of erythromycin resistance among Streptococci isolates (63.5%) was similar to that of Staphylococci (65.5%) as shown in Table 3.2 and Figure 3.1. However, higher percentage of clindamycin resistant isolates was found among Streptococci (48.1%) in comparison with Staphylococci (20.5%). Difference in clindamycin resistant between the Staphylococci and Streptococci were of no significant value (***P***= 0.499). MIC values for both erythromycin and clindamycin among resistant Streptococci isolates ranged from 1 to ≥128µg/ml. *S. pyogenes* showed 100% rate of resistant to erythromycin and clindamycin while none of *S. pneumoniae* isolates showed resistance to both antibiotics.

Frequency of erythromycin resistance (70.6%) among methecillin resistant Staphylococci isolates was insignificantly (***P***= 0.095) higher than that among methecillin susceptible isolates (59.3%) as shown in Table 3.3. Similarly, clindamycin resistance among methecillin resistant Staphylococci (23.9%) was higher than that among methecillin susceptible isolates (16.5%, ***P***= 0.199).

Table 3.3. Erythromycin and clindamycin resistance among methecillin resistant and susceptible Staphylococci.

Bacterial species	Total No	Methecillin resistant			Methecillin susceptible		
		Isolates No	Erythromycin resistant (%)	Clindamycin resistant (%)	Isolates No	Erythromycin resistant (%)	Clindamycin resistant (%)
Staphylococci	200	109	77(70.6)	26(23.9)	91	54(59.3)	15(16.5)
S. auerus	187	102	73(71.6)	25(24.5)	85	48(56.5)	14(16.5)
CONS*	13	7	4 (57.1)	1(14.3)	6	6(100)	1(16.7)
S.epidermidis	12	6	4(66.7)	1(16.7)	6	6(100)	1(16.7)
S.saprophyticus	1	1	0(0)	0(0)	0	-	-

*CONS, coagulase negative Staphylococci

Relatively high percentages of erythromycin resistance were found among Staphylococci and Streptococci isolates obtained from hospitals and private laboratories included in the present study (Table 3.4). Staphylococci isolates from Al-Watani hospital showed the highest resistance rate (85.7%) for both erythromycin and clindamycin. This rate was significantly higher than that found among isolates collected from Rafedia hospital (***P***=0.000). On the other hand, clindamycin resistance among Streptococci isolates obtained from the New Technology laboratory was the highest (61.5%). The differences were without significant value.

Table 3.4: Clinical data of erythromycin and/or clindamycin resistant Staphylococci and Streptococci isolates.

Variable	Staphylococci			Streptococci		
	No. isolates*	E R* (%)	DA R* (%)	No. isolates	E R (%)	DA R (%)
Source						
Rafidia hospital	140	90 (64.3)	22(15.7)	20	8(40)	7(35)
New Technology laboratory	9	5 (55.6)	2(22.2)	26	20 (76.9)	16(61.5)
Nablus Specialty hospital	21	12 (57.1)	6(28.6)	5	4(80)	2(40)
Al-Arabi Specialty hospital	18	14 (77.8)	3(16.7)	1	1(100)	0(0)
Al-Watani hospital	7	6 (85.7)	6(85.7)	0	0(0)	0(0)
Medicare-laboratory	5	4 (80)	2(40)	0	0(0)	0(0)
Units						
Out patients	56	41(73.2)	14(25)	36	26 (72.2)	22(61.1)
Inpatients	144	90(62.5)	27(18.8)	16	7(43.8)	3(18.8)
General surgery	32	12 (37.5)	1(3.1)	5	1(20)	1(20)
Emergency	17	9(52.9)	0(0)	3	2(66.7)	2(66.7)
Pediatrics	16	11(68.8)	4(25)	2	1(50)	0(0)
Burns	16	10(62.5)	3(18.8)	0	0(0)	0(0)
Neonates	14	12(85.7)	5(35.7)	1	0(0)	0(0)
Urology	14	8(57.1)	3(21.4)	0	0(0)	0(0)
ICU*	9	6(66.7)	4(44.4)	2	0(0)	0(0)
Internal medicine	13	10(76.9)	4(30.8)	2	2(100)	0(0)
Orthopedic	7	6(85.7)	1(14.3)	0	0(0)	0(0)
Gynecology	6	6(100)	2(33.3)	1	1(100)	0(0)
Specimen						
Wound swab	126	75(59.5)	19(15.1)	17	10(58.8)	10(58.8)
Urine	23	18(78.3)	7(30.4)	19	15(79)	12(63.2)
Blood	8	8(100)	2(25)	2	1(50)	0(0)
Sputum	6	4(66.7)	4(66.7)	3	2(66.7)	0(0)
Nasal swab	8	8(100)	2(25)	0	0(0)	0(0)
Fluid	5	2(40)	2(40)	1	1(100)	1(100)
Throat swab	1	1(100)	0(0)	5	2(40)	0(0)
Vaginal swab	2	2(100)	1(50)	3	2(66.7)	2(66.7)
Umbilical swab	5	4(80)	2(40)	0	0(0)	0(0)
Ear swab	2	0(0)	0(0)	1	0(0)	0(0)
Semen	3	2(66.7)	0(0)	0	0(0)	0(0)
Skin	2	1(50)	0(0)	0	0(0)	0(0)
Burn swab	2	1(50)	0(0)	0	0(0)	0(0)
Tissue	2	1(50)	1(50)	0	0(0)	0(0)
CVP*	1	1(100)	0(0)	0	0(0)	0(0)
Chest swab	1	1(100)	0(0)	0	0(0)	0(0)

Table 3.4 (continued)

Variable	Staphylococci			Streptococci		
	No. isolates*	E R* (%)	DA R* (%)	No. isolates	E R (%)	DA R (%)
CSF*	0	0(0)	0(0)	1	0(0)	0(0)
Pus	1	0(0)	0(0)	0	0(0)	0(0)
Drain	1	1(100)	1(100)	0	0(0)	0(0)
Breast discharge	1	1(100)	0(0)	0	0(0)	0(0)
Sex						
Male	113	78(69.9)	26(26.5)	19	12(63.2)	9(47.4)
Female	87	53(60.9)	15(18.4)	33	21(63.6)	16(48.5)
Total	200	131(65.5)	41 (20.5)	52	33(63.5)	25(48.1)

* No. isolates, number of isolates; E R, Erythromycin resistant; DA R, Clindamycin resistant; CVP, central venous catheter**;** CSF, Cerebrospinal Fluid.

With respect to erythromycin resistance, Staphylococcal strains isolated from gynecology unit were with the highest frequency (100%) compared to isolates from other departments as well as outpatients' isolates. Frequency differences were significant in comparison with those isolates obtained from outpatients and patients of general surgery, emergency, pediatrics and burns units (***P***=0.000). On the other hand, clindamycin highest frequency of resistance was found in intensive care unit (44.4%), which was also significantly higher than that of emergency department (***P***= 0.001).

Pronounced resistance against erythromycin and clindamycin was found among Streptococcal bacteria isolated from outpatients (Table 3.4).

Among the most commonly encountered specimens in the present study, resistance to erythromycin was highest in Staphylococci bacteria isolates of blood and nasal swabs (100% both) followed by urine isolates (78%). The resistance of bacterial isolates of blood and nasal swabs, were significantly higher than that found among wound swabs (***P***=0.000).

Resistance to clindamycin was highest in Staphylococci bacteria isolated from sputum (66.7%) followed by urine (30.4%). It was also found that out of 19 Streptococci bacteria isolated from urine specimen, 15 (79%) and 12 (63%) were resistant to erythromycin and clindamycin, respectively. Among Streptococci, no significance association between specimen type and resistance rate was detected.

Analysis of antibiotic resistance for Staphylococci isolates obtained from males and females showed that erythromycin resistance is slightly higher among male isolates (69.9%) compared to females (60.9 %) and the frequency of resistance were very similar in both genders in the case of Streptococci isolates (Table 3.4). It was also found that clindamycin resistance among Staphylococci was slightly higher in male's isolates (26.5%), while Streptococci bacteria showed slightly higher resistance in isolates obtained from females (48.5%) as shown in Table 3.4.

The mean age of patients infected by erythromycin resistant Staphylococci (23 years) and Streptococci (23.7 years) isolates was slightly different from that of patients with erythromycin susceptible Staphylococci (25.1 year) and Streptococci (20 years) isolates. No significant association was found. However, variation of clindamycin resistance was clear with respect to mean age of patients. Clindamycin resistant Staphylococci strains were isolated from patients with mean age (31.9 year), higher than that of clindamycin susceptible Staphylococci isolates (21.6 year). In addition, clindamycin resistant Streptococci isolates were isolated from

patients with mean age higher (31.3 year) than that of clindamycin susceptible Streptococci isolates (25.5 year). Such differences were of no significance for the tested antibiotics.

Table 3.5 below displays frequencies of antibiotics resistance of both Staphylococci and Streptococci isolates among different age groups. In Staphylococci bacterial isolates, erythromycin resistance was relatively high in all age groups (Figure 3.2), however, it was highest among age groups 0-2 years (74.5%) and age group >65 years (75%). In a similar manner, clindamycin resistance among Staphylococci was highest in bacteria isolated from patients >65 years (50%), which was also significantly higher than that of 3-14 year age group (***P***= 0.007). Among age groups with abundant Streptococcal isolates, erythromycin (80%) and clindamycin (60%) resistance were found in age group 40-65 years.

Table 3.5 Distribution of erythromycin and clindamycin resistant isolates among different age groups.

Age (years)	Total	Staphylococci			Streptococci		
		No*	Er R*(%)	DA R* (%)	No	Er R(%)	DA R(%)
0-2	55	51	38 (74.5)	13(25.5)	4	2(50)	0(0)
3-14	32	29	15 (51.7)	1(3.5)	3	1(33.3)	1(33.3)
15-39	56	36	22 (61.1)	3(8.3)	20	15(75)	12(60)
40-65	41	31	18 (58.1)	8(25.8)	10	8(80)	6(60)
>65	14	12	9 (75)	6(50)	2	2(100)	1(50)
un known	54	41	29 (70.7)	10(24.4)	13	5(38.5)	5(38.5)

*No, number; Er R, Erythromycin resistant; DA R, Clindamycin resistant.

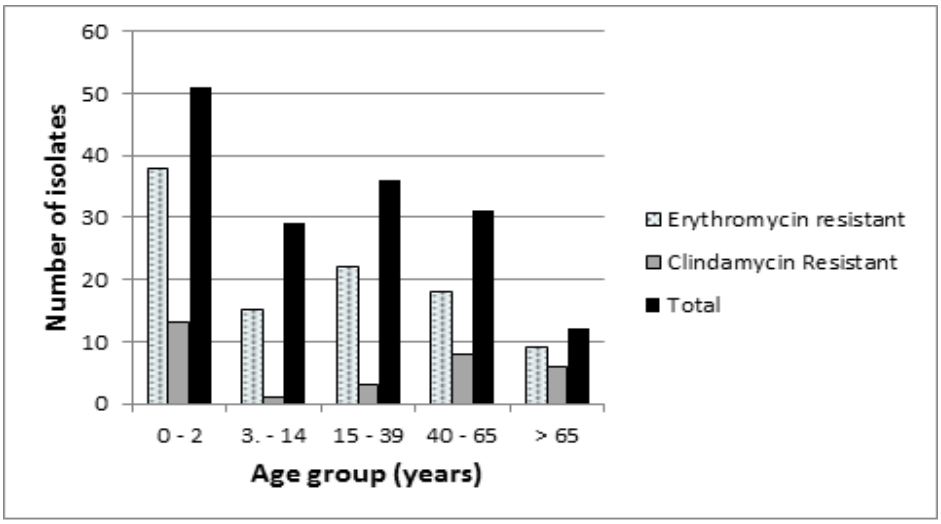

Figure 3.2 Distribution of resistant Staphylococcal isolates in different age groups.

3.3 Mechanisms of resistance to MLS_B

MIC values for erythromycin and clindamycin, erythromycin-clindamycin induction test and detection of resistance genes by PCR were combined to predict the most probable mechanism of resistance. The results indicate that resistance of Staphylococci isolates against erythromycin appear to be mediated by efflux mechanism (MS phenotype, 50.4%) and target site modification (MLS_B phenotypes, 49.6%) as shown in Table 3.6. Enzymatic inactivation of macrolides appears to have limited participation in the erythromycin resistance as predicted by the absence of enzymatic inactivating gene (*ere*) in all representative Staphylococci isolates examined for this purpose. In addition, detection of efflux gene *(msr)* in all examined isolates possessing resistance to erythromycin and susceptibility to clindamycin and negative for the induction test, indirectly confirms that enzymatic inactivation is rare in Gram-positive bacteria. Staphylococci isolates with target modification mode of resistance

expressed MLS_B phenotype constitutively and inducible in 61.5% and 38.5% of the isolates, respectively. The above findings indicate that a considerable proportion of erythromycin resistant isolates (19.1%) exhibited inducible MLS_B. In *S. aureus* MLS_B phenotypes (51.2% of resistant isolates) was detected more frequently than CONS (30%) and the situation was reversed with respect to MS (Table 3.6). No significant association was found between bacterial type and phenotype of resistance.

Table 3.6 Resistance phenotypes among Staphylococcal isolates.

Isolate	Erythromycin resistant isolates	Phenotype of resistance		
		MLS_B-con(%)*	**MLS_B-in(%)***	**MS(%)***
Staphylococci	131	40 (30.5)	25 (19.1)	66 (50.4)
s. auerus	121	38 (31.4)	24 (19.8)	59 (48.8)
CONS*	10	2 (20)	1 (10)	7 (70)
S. epidermidis	10	2 (20)	1 (10)	7 (70)
S.saprophyticus	0	0 (0)	0 (0)	0 (0)

*con, constitutive; in, inducible; CONS, Coagulase Negative Staphylococci

Data presented in Table 3.7 shows the phenotypes of resistance to erythromycin among Streptococci isolates. The finding of 75.8% of the studied isolates possessing MLS_B resistance phenotype indicates that resistance to erythromycin is most commonly mediated by target modification. However, efflux mechanism of resistance (M phenotype) was detected in 8 (24.2%) of the isolates. No significant association was found between Streptococcal bacterial species and resistance's phenotype.

Table 3.7 Phenotypes of resistance among Streptococcal isolates.

Isolate type	Erythromycin resistant isolates	Phenotype of resistance	
		MLS_B (Con or In)*%	**M%**
Streptococci	33	25(75.8)	8(24.2)
S. agalactiae	26	20**(76.9)	6(23.1)
S.viridans	5	3(60)	2(40)
S.pneumoniae	0	0(0)	0(0)
S.pyogenes	2	2(100)	0(0)

*Con or In, constitutive or inducible

**Including one isolate with positive induction test

3.4 Detection of resistant genes

A representative sample of erythromycin resistant isolates (47) was examined for presence of erythromycin resistance genes. Initially, multiplex- PCR was carried out with a reaction mixture containing more than 1 pair of primers (*ermB* and *ermA*; *ermB*, *ermA* and *msr*). Following this procedure did not yield good products. However, better sensitivity and product yield was obtained using single pair of primers in a reaction mix containing a higher $MgCl_2$ concentration (4mM). PCR products of representative samples are shown in Figures 3.3 and 3.4. The results showed that genes of *ermC* and *ermA* were detected in 11 and 9 Staphylococci isolates, respectively. On the other hand *ermB* was detected in 9 cases and *ermC* in only one case among Streptococci isolates (Table 3.8). It was also found that *ermA* and *ermC* were predominant among

Staphylococci isolates while *ermB* was predominant among Streptococci isolates.

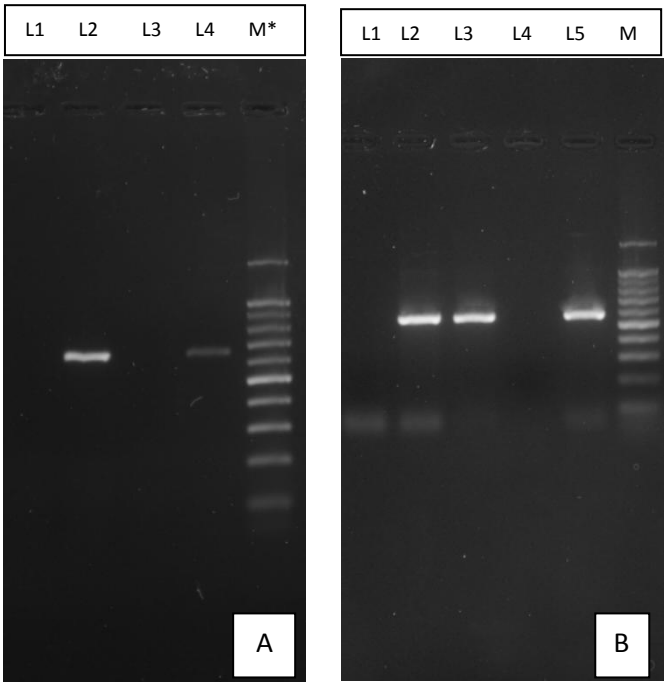

Figure 3.3. Amplified PCR products of *erm* genes.

A. *ermA* and *ermB* separately: Lane (L)1 is a negative *ermB* result; L2, *ermB* positive; L3, *ermA* negative; L4, *ermA* positive. **B. *ermC*:** L1 and L4, *ermC* negative; L2, L3 and L5, *ermC* positive; M: 100 bp ladder.

Efflux gene (*msr*) was detected in all 18 examined Staphylococci isolates exhibiting resistant to erythromycin, susceptible to clindamycin, and negative erythromycin-clindamycin induction test. All tested isolates were also negative with respect to the enzymatic inactivation *ere* gene. Such findings confirm that these isolates possess the MS phenotype. In addition, *msr* gene was detected in 2 isolates possessing the MLS_B phenotype.

Efflux gene (*mef*) was detected in 2 streptococcus isolates exhibiting the M phenotype and in one isolates exhibiting the MLS_B phenotype.

Enzymatic inactivating gene (*ere*) was only detected in 1 isolate exhibiting MLS_B phenotype in association with other target modifying resistant genes (*ermC* and *ermB*).

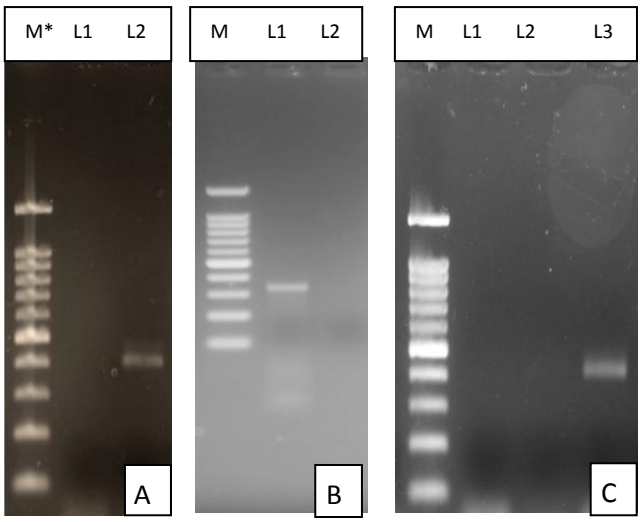

Figure 3.4. Amplified PCR products for *msr, mef, ere* genes

A *msr gene:* L1, *msr* negative; L2, *msr* positive

B. *mef* gene: L1, *mef* positive; L2, *mef* negative

C. *ere* gene: L1 and L2, *ere* negative; Lane 3, *ere* positive

*M, 100 bp ladder

Table 3.8 Genes of macrolide resistance found in examined Staphylococcal and Streptococcal isolates.

Type of bacteria and resistance phenotype	Examined isolates	Detected Gene(%)					
		me,f*	***ere****	***ermC****	***ermB****	***ermA****	***msr****
Staphylococci	36	0(0)	0(0)	11(30.6)	0(0)	9(25)	20(55.6)
MLSb constitutive	7	0(0)	0(0)	7(100)	0(0)	0(0)	1(14.3)
MLSb inducible	11	0(0)	0(0)	4(36.4)	0(0)	8(72.7)	1(9.1)
MS	18	0(0)	0(0)	0(0)	0(0)	1(5.55)	18(100)
S.aureus	34	0(0)	0(0)	9(26.5)	0(0)	9(26.5)	19(55.9)
MLSb constitutive	5	0(0)	0(0)	5(100)	0(0)	0(0)	0(0)
MLSb inducible	11	0(0)	0(0)	4(36.4)	0(0)	8(72.7)	1(9.1)
MS	18	0(0)	0(0)	0(0)	0(0)	1(5.55)	18(100)
CONS	2	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
MLSb constitutive	2	0(0)	0(0)	2(100)	0(0)	0(0)	1(50)
Streptococci	11	3(27.3)	1(9.1)	1(9.1)	9(81.8)	0(0)	0(0)
MLS(C orI)	8	1(12.5)	1(12.5)	1(12.5)	8(100)	0(0)	0(0)
M	3	2(66.7)	0(0)	0(0)	1(33.3)	0(0)	0(0)

* *erm*, erythromycin ribosome methylase; *msr*, macrolide-streptogramin B resistance gene; *mef*, macrolide efflux gene; CONS, coagulase negative Staphylococci.

In staphylococcal isolate, presence of multiple resistant determinant genes was found. The gene *msr* was found in combination with *ermC* in 2 isolates and with *ermA* in one isolate. In addition, both genes (*ermC and ermA)* were found in one isolate. On the other hand, among Streptococcal isolates, *ermB* gene was found in association with *mef* genes in a single isolate. *ermB* gene was also found in association with *ermC* and *ere* genes in another different isolate.

3.5 Nosocomial infection

Evidence of nosocomial infection (concluded from isolation of bacteria after 48 hour of hospitalization) was found in 36 cases (24 were erythromycin resistant and 14 clindamycin resistant). Findings on resistant phenotypes and source of isolates for several samples (Rafidia hospital: pediatric 2, urology 2 and burns department 3) indicates relatedness of isolates and their role of nosocomial infections. To confirm this assumption, further molecular typing for these isolate is required.

Chapter Four
Discussion

Resistance to antimicrobial drugs is a worldwide problem and recognized as a threat to public health and patient safety. It reduces the available treatment options and causes increased morbidity and mortality as well as increased costs due to failure of empirical antimicrobial therapy. It is also accepted that improper use of antimicrobials is behind the increased selection pressure for antimicrobial resistance. Implementations of national programs, which monitor antimicrobial use and resistance have been shown to be an efficient approach for preserving the effectiveness of antimicrobial agents in many countries (126, 127).

In the current study, a relatively high frequency of erythromycin resistance among Staphylococci isolates (65.5%) was found. Resistance to erythromycin was more frequent in coagulase negative Staphylococci (CONS) than in coagulase positive Staphylococci (COPS), which were 76.9% *and* 64.7%, respectively. In a Turkish study (128), 59.2% of Staphylococci isolates collected during the period 2003 to 2005 were resistant to erythromycin. This study reported similar finding as they found that resistant rate to erythromycin in CONS (69.8%) was more than that observed among COPS isolates (49.6%). Other studies (129,130, 131, 132) also reported that, CONS were more likely to be erythromycin resistant than *S. aureus*. This may be explained by presence of CONS as normal flora in the patients before causing infection, a situation that allows longer exposure periods to antibiotics and consequently better condition for natural selection of resistance. Studies from Europe and USA, carried during the period 1996-1999 (133, 129, 134), reported an incidence of

resistance to erythromycin in *S. aureus* strains ranging from 13-30% in Europe and 20-50% in the USA. Lower resistant rates to erythromycin in these studies compared to our findings are most likely due to time factor. Such differences in resistance rates emphasize the importance of continuous monitoring of drug resistance development among bacterial strains.

In the present study, erythromycin resistance rate (70.6%) among methecillin resistant Staphylococci isolates was insignificantly higher than that among methecillin susceptible isolates (59.3%). Similar previous studies reported that 76.8% of methecillin resistant isolates were resistant to erythromycin and 28.6% of methecillin susceptible isolates were resistant to erythromycin (128). Higher erythromycin resistant rate among methecillin resistant Staphylococci has been linked to the presence of erythromycin resistant genes conserved in *mec* DNA (135). However, methecillin resistant Staphylococci strains that have susceptibility to erythromycin, as well as methecillin susceptible isolates with erythromycin resistance were reported (136, 128).

Frequency of erythromycin resistance among Streptococci strains in the current study was relatively high (63.5%). Among the most commonly encountered Streptococci species, resistance rates of *S. agalactiae* and viridians Streptococci were 78.8 and 35.7%, respectively. Lower resistance rate to erythromycin among *S. agalactiae isolates* (16.3%) was reported (137). However, erythromycin resistance among viridans

Streptococci in our study was very close to that (36%) reported by Helena Seppälä et al (138).

Prevalence of clindamycin resistance rate among Staphylococci in our study (20.5%) was lower than that of erythromycin (65.5%). This can be attributed to the induction capacity of erythromycin for methylase enzyme production that performs ribosomal modification as a mode of resistance. A higher rate of clindamycin resistance among Staphylococci (46.97%) was reported in India compared to our findings (139). Furthermore, in present study, Streptococci expressed higher resistance rate to clindamycin (48.1%) than Staphylococci. This can be explained by the fact that clindamycin is an inducer at different degree of *ermB* gene for methylase enzyme production in Streptococci but not in Staphylococci (90). Resistant to clindamycin in different species of Streptococci were reported to range from 0.8% to 30.6% (140, 137, 59).

The finding of 2 lincosamide resistant Staphylococci isolates that were not resistant to macrolides indicates the presence of other resistant phenotypes. Low prevalence of this phenotype was also reported by Leclereq et al (58).

In the present study, resistance of Staphylococci isolates to erythromycin appears to be mediated by both efflux (MS phenotype) and target site modification (MLS_B phenotypes) mechanisms as they were detected in 50.4% and 49.6% of resistant strains, respectively. These mechanisms were also reported to be behind erythromycin resistance

among Staphylococci isolates in India, where 70.5% were MLS_B phenotype and 29.5% were of MS phenotype (139). The study also reported that among the erythromycin resistant isolates, constitutive MLS_B resistance was expressed in 46.97% while inducible clindamycin resistance MLS_B expressed only in 23.48% of the isolates. In erythromycin resistant isolates of the present study, 30.5% expressed MLS_B phenotype constitutively and 19.1% inducibly. Thus, a considerable proportion of erythromycin resistant isolates exhibited inducible MLS_B phenotype. These isolates will appear susceptible to clindamycin, in disk diffusion method, and will be at a high risk of conversion from inducible to constitutive MLS_B phenotype in vivo. As a result of conversion one should expect clindamycin medication failure (88, 85). Thus, simple laboratory testing (erythromycin-clindamycin induction test) can separate strains with genetic potential (i.e., the presence of *erm* genes) to become resistant during therapy from strains that are fully susceptible to clindamycin.

We found that most (75.8%) of erythromycin resistant Streptococci isolates possessed MLS_B phenotype. However, efflux mechanism of resistance (M phenotype) was detected in 24.2% of resistant isolates. Among the commonly isolated Streptococcal species, *streptococcus agalactiae* possessed MLS_B and M phenotype in 76.9% and 23.1% of erythromycin resistant isolates, respectively. Similar findings on phenotype frequency of *streptococcus agalactiae* isolates were reported (137). In the current study viridans Streptococci also predominantly expressed MLS_B phenotype (60%) and to lesser degree M phenotype (40%). This finding is

contradictory to that reported by Cerda Zolezzi et al (59) as M resistant phenotype was more prevalent (60%) than MLS_B phenotype (40%). *S. pyogenes* represented by two isolates showed the MLS_B phenotype. Among erythromycin resistant *S.pyogenes* isolates, M phenotype was more prominent compared to MLS_B (140). Variations in erythromycin and clindamycin resistant frequencies as well as resistant phenotypes in different parts of the world are expected to occur due to time factor, compliance and use of antibiotics and outbreaks of a resistant strain in clinical settings during study periods.

The lack of outer membrane of cell-wall in Gram-positive bacteria (17, 65) causes diffusion of antibiotic modifying enzymes to surrounding media and prevents their concentration as in Gram-negative bacteria. Therefore, enzymatic inactivation is rarely reported as a mechanism of resistance in Gram-positive cocci (141, 120, 142, 143). Similarly, in the present study, enzymatic inactivation of macrolides was predicted to have limited participation in the erythromycin resistance. The absence of amplified PCR products for enzymatic inactivating gene (*ere*) in all 36 examined Staphylococci isolates is in support of the limited role of enzymatic participation. In Streptococci isolates, *ere* gene was detected in combination with *ermB* and *ermC* genes in one single isolate with MLS_B phenotype of resistance (target modification). Such findings are in support that target modification is behind resistance in this isolate. In addition, detection of efflux (*msr*) gene in all examined Staphylococci isolates possessing resistance to erythromycin, susceptibility to clindamycin and

non-inducible resistance to clindamycin indirectly confirms that enzymatic inactivation is rare in Gram-positive bacteria.

In the current study, out of 36 examined Staphylococci isolates, 9 isolates (25%) possessed *ermA*, 11 (30.6%) *ermC* , and 20 (55.6%) *msr*. Both *ermB* and *ere* were not detected. A low prevalence of *ermB* among Staphylococci was also recorded in earlier studies (144, 145). Absence of *ere* gene in *S. aureus* isolates was also reported by Schmitz et al (120),

Among the 11 examined erythromycin resistant Streptococci isolates, *ermB* gene was detected in 9 (81.8 % of examined strains); of which 7 were *S. agalactiae* and were 2 viridans isolates. In addition, *ermC* was detected in one *S. agalactiae* isolate. The efflux gene (*mef*) was detected is 3 streptococcus isolates (2 agalactiae and 1 in viridans) of which two were with M phenotype and 1 isolate with MLS_B phenotype. Enzymatic inactivating gene (*ere*) was only detected in one isolate exhibiting MLS_B phenotype in association with other resistant determinants (*ermC* and *ermB*). Zolezzi et (100), detected *ermB* in all erythromycin resistant viridians Streptococci strains possessing MLS_B phenotype, either alone or in combination with *mef* gene. Among *S. agalactiae ermB* gene was detected 96% of isolates with MLS_B (137). and *mef* gene was detected in all isolates with the M phenotype.

In the current study, variations in frequencies of antibiotic resistance among different studied centers seems to be attributed to hospital unit type. The finding of higher percentage of erythromycin and clindamycin

resistance among Staphylococci in Al-Watani hospital could be attributed to the fact that this hospital is specialized mostly for internal medicine where the patient could be suffering of severe infection, while Rafedia hospital is a surgical hospital and admitted patients most likely enrolled for sugary and not for treatment of infections.

The finding of significantly higher frequencies of erythromycin resistance among Staphylococci isolates recovered from gynecology unit in comparison to other units could be attributed to the fact that most of the women admitted to this department were pregnant or were admitted for delivery. This group of women are more sensitive to bacterial infection due to modulated immunity (146, 147), resulting in prolong bacterial infection periods allowing the possibility of development of resistance against different antibiotics.

Among the most commonly encountered specimens in the present study, resistance to erythromycin was highest in Staphylococci bacteria isolated from blood and nasal swabs (100% both), which were significantly higher than that of wound swabs (***P***=0.000). In addition, resistance to clindamycin was highest in Staphylococci isolated from sputum (66.7%). Many of the cases of bacteremia and septicemia are complications after primary infection in sites other than blood circulation (148, 149). Usually a patient receives treatment before these complications. Therefore, the bacterial strains reaching blood is expected to be resistant to antibiotics used for the treatment of primary infections. The inside of the nasal cavity

and respiratory tract are known for their poor blood circulation. This is expected to decrease exposure of bacteria to immune system of the host and decrease in the exposure dose of antibiotic to bacteria. Under these conditions bacteria will have the advantage to develop antibiotic resistance. This might explains the high resistance rate to both studied antibiotics among Staphylococci strains isolated from nasal and sputum specimens. High prevalence of multidrug resistance (non-susceptibility to ≥ four antimicrobial classes) in MRSA nasal isolates was also reported by Meghan et al (150). No Streptococci bacteria was found among nasal swabs and the number was limited among sputum isolates, thus, it was difficult to find similar relation between patterns of resistance and specimen types as in Staphylococci.

With respect to age groups, erythromycin resistance showed the highest rate among Staphylococci isolates recovered from 0-2 years and >65 years. This could be due to the capacity of the immune system in these age groups. The findings of very high resistant rates to erythromycin among Staphylococci isolated from neonates (90% of *S. epidermidis* and 100% of *S. haemolyticus* were resistant) are in agreement with our findings regarding 0-2 age group. (151). On the other hand, the findings of Adam et al (152) on resistance of *S. aureus*, *S. pneumoniae* and other pathogens to antibiotics (methecillin, clindamycin and claritromycin) are consistent with our findings among age group >65 years.

Recommendations and concluding remarks

- The current study clearly indicates the presence of high macrolide resistant rates among bacterial isolates collected from various clinical settings. In addition, a considerable proportion of resistance was due to inducible phenotype, a situation that requires more attention by medical staff when deciding a suitable antibiotic. In our situation it seems to be essential to carry out the induction test before any decision for clindamycin prescription. It is also essential to have in mind variations of resistance rate among various age groups, specimen type and pregnant women in particular.
- In conclusion, it seems essential that the concern governmental bodies pay more attention for monitoring resistance rates in the various clinical setting in the country in order to adopt the best treatment policy.

References

1. National Nosocomial Infections Surveillance System. 2003. **National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2003**. Am J Infect Control 31:481-98.

2. Jones RN. 2003. **Global epidemiology of antimicrobial resistance among community-acquired and nosocomial pathogens: a five-year summary from the SENTRY Antimicrobial Surveillance Program (1997–2001).** Semin Respir Crit Care Med 24:121-34.

3. Bruinsma N, Kristinsson KG, Bronzwaer S, et al. 2004. **Trends of penicillin and erythromycin resistance among invasive Streptococcus pneumoniae in Europe.** Antimicrob Chemother J 54:1045-50.

4. National Nosocomial Infections Surveillance System. 2004. **National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004.** Am J Infect Control 32:470–485.

5. Chambers HF. 2001. **The changing epidemiology of *Staphylococcus aureus*?** Emerg Infect Dis 7:178 –182.

6. Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. 1999. **Nosocomial bloodstream infections in United States hospitals: a 3-year analysis.** Clin Infect Dis 29:239 –244.

7. Jones RN, Kirby JT, Beach ML, Biedenbach DJ, Pfaller MA. 2002. **Geographic variations in activity of broad-spectrum beta-lactams against *Pseudomonas aeruginosa*: summary of the worldwide SENTRY Antimicrobial Surveillance Program (1997–2000).** Diagn Microbiol Infect Dis 43:239 –243.

8. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. 2003. **Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States, 1998 to 2001.** Antimicrob Agents Chemother 47:1681–1688.

9. Martone WJ. 1998**. Spread of vancomycin-resistant enterococci: why did it happen in the United States?** Infect Control Hosp Epidemiol 19: 539–545.

10. Kang CI, Kim SH, Park WB, et al. 2005. **Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome.** Antimicrob Agents Chemother 49:760 –766.

11. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. 2000. **The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting.** Chest 118:146 –155.

12. Kloos WE, Bannerman TL. 1994. **Update on clinical significance of coagulase-negative staphylococci**. Clin Micribiol Rev 7: 117-140.

13. Pfaller MA, Herwaldt LA. 1988. **Laboratory, clinical and epidemiological aspects of coagulase-negative staphylococci**. Clin Microbiol Rev 1:281-299.

14. Rupp ME, Archer GL. 1994. **Coagulase-negative staphylococci: pathogens associated with medical progress.** Clin Infect Dis 19:231-245.

15. Stevenson KB. 2005. **Methicillin-resistant *Staphylococcus aureus* and vancomycin- resistant enterococci in rural communities, Western United States.** Emerg Infect Dis11:895–903.

16.Masterton R. 2008. **The Importance and Future of Antimicrobial Surveillance Studies.** Clin Infect Dis 47 (Supplement 1): S21-S31.

17.Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Wood G. **Color atlas and textbook of diagnostic microbiology**. 6 th edn. Philadelphia: A Wolters Kluwer company; 2006. 623-671.

18. Brooks GF, Butel JS, Morse SA. **Medical Microbiology**. 23 edition. New York: Mc. Graw Hill; 2004. 223-247.

19. Brumfitt W, Hamilton-Miller J. 1989. **Methicillin-resistant Staphylococcus aureus**. N Engl J Med 320: 1188-1196.

20.Sheagren JN. 1984. **Staphylococcus aureus: the persistent pathogen**. N Engl J Med 310: 1368-1442.

21. Schaberg DR, Culver DH, Gaines RP. 1991. **Major trends in the microbial etiology of nosocomial infection**. Am J Med 91(suppl. 3B): 725-735.

22. Marchant EA, Boyce GK, Sadarangani M, Lavoie P M. 2013. **Neonatal Sepsis due to Coagulase-Negative Staphylococci**. Clin Develop Immun 2013: 10.

23. Archer GL, Climo MW. 1994. **Antimicrobial susceptibility of coagulase-negative staphylococci**. Antimicrob Agent Chemother 38: 2231-2237.

24. Clewell DB. 1981. **Plasmids, Drug Resistance, and Gene Transfer in the Genus Streptococcus**. Micrbiol Rev 45: 409-436.

25. Bisno AL. *Streptococcus pyogeges*. In Mandell, Douglas and Bennett□s **Principles and Practice of Infectious Diseases**, 4th edn, (Mandell, G. L., Bennett, J. E. and Dolin, R., Eds),. New York: Churchill Livingstone; 1995. 1786-1799.

26. Kennedy HF, Gemmell CG, Bagg J, Gibson BES, Michie J. R. 2001. **Antimicribial susceptibility of blood cultures isolates of viridans group streptococci: relationship to a change in empirical antibiotic therapy in febrile neutropenia**. J Antimicrob Chemother 47: 693-696.

27. Douglas CWI, Heath J, Hampton K, Preston FE. 1993. **Identity of viridans streptococci isolated from cases of infective endocarditis**. J Med Microbiol 39: 179-182.

28. Schuchat A. 1999. **Group B Streptococcus**. Lancet 353: 51–6.

29. Centers of Disease Control. 1996. **Prevention of perinatal group B streptococcal disease: a public health perspective**. Morb Mort W Rep 45: 1–24.

30. Baker JC, Barrett FF, Gordon RC, Yow MD. 1973. **Suppurative meningitis due to streptococci of Lancefield group B; a study of 33 infants.** J Pediatr 82:724.

31. Yew X. 1974. **Group B streptococci: a serious threat to the neonate**. J Am Med Asoc 230: 1177-1178.

32. Chang SC, Chen YC, Luh KT, Hsieh WC. 1995. **Macrolide resistance of common bacteria isolated from Taiwan**. Diagn Microbiol Infect Dis 23, 147–54.

33. Duval J. 1985. **Evolution and epidemiology of MLS resistance.** J AntimicrobChemother 16, Suppl. A, 137–49.

34. Sanchez M, Flint K, Jones RN. 1993. **Occurrence of macrolide-lincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. Is false susceptibility to new**

macrolides and clindamycin a contemporary clinical and in vitro testing problem? Diagn Microbiol Infect Dis 16, 205–3.

35. Thakker-Varia S, Jenssen WD, Moon-McDermott L, Weinstein MP, Dubin DT. 1987. **Molecular epidemiology of macrolide-lincosamide-streptogramin B resistance in Staphylococcus aureus and coagulase-negative staphylococci**. Antimicrob Agent Chemother 31, 735–43.

36. Elks J, Ganellin CR. (Eds). **Dictionary of Drugs.** London: Chapman and Hall; 1991.

37. Bryskier AJ, Butzler JP, Neu HC Tulkens PM. eds. **Macrolides: Chemistry, Pharmacology and Clinical Uses.** Paris: ArnetteBlackwell; 1993. 379–381.

38. Reynolds JEF. (Ed.), **Martindale, The Extra Pharmacopoeia,** 30th ed., London: The Pharmaceutical Press; 1993.

39. Morar M, Bhullar K, Hughes DW, Junop M, Gerard D. December 9, 2009. **Wright Structure and Mechanism of the Lincosamide Antibiotic Adenylyltransferase LinB**. Structure 17: 1649–1659.

40. Magerlein B J, Birkenmeyer RD, Herr RR, Kagan F. 1967. **Lincomycin. V. Amino acid fragment**. J Am Chem Soc 89: 2459-2464.

41. Schroeder W, Bannister B, Hoeksema H. 1967. **Lincomycin. III. The structure and stereochemistry of the carbohydrate moiety**. J Am Chem Soc 89: 2448-2453.

42. Slomp G, MacKellar FA. 1967. **Lincomycin. IV. Nuclear magnetic resonance studies on the structure of lincomycin**, **its degradation products, and some analogs.** J Am Chem Soc 89: 2454- 2459.

43. Chung ST, Manis JJ, Wethy S J, Patt TE, Witz DF, Wolf H J, Wovcha M G. **Fefmentation, Biosynthesis, and Molecular Genetics of Lincomycin in Biotechnology of antibiotics**. 2nd edn. Edited by Strohl, W. R. New York: Marcel Dekker, INC; 1997. 165-186.

44. Weisblum B. 1995. **Erythromycin Resistance by Ribosome Modification**. AntimicrobAgent Chemother 39: 577–585

45. Porse BT, Garrett RA. 1999. **Sites of interaction of streptogramin A and B antibiotics in the peptidyl transferase loop of 23 S rRNA and the synergism of their inhibitory mechanisms**. J Mol Biol 286: 375–387.

46. Schlunzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, et al. 2001. **Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria**. Nature 413: 814–821.

47. Hansen JL, Ippolito JA, Ban N, Nissen P, Moore PB, Steitz TA. 2002. **The structures of four macrolide antibiotics bound to the large ribosomal subunit.** Mol Cell 10: 117–128.

48. Vazquez D. 1966. **Binding of chloramphenicol to ribosomes. The effect of a number of antibiotics**. Biochim Biophys Acta 114: 277–288.

49. Vazquez D, Monro RE. 1967. **Effects of some inhibitors of protein synthesis on the binding of aminoacyl tRNA to ribosomal subunits**. Biochim Biophys Acta 142: 155–173.

50. Gale EF, Cundliff e E, Reynolds PE, Richmond MH, Waring MJ. **The molecular basis of antibiotic action,** 2nd ed, New York: John Wiley & Sons, Inc; 1981. p. 478-480.

51.Monro RE, Fernandez-Munoz R, Celma ML, Vazquez D. **Mode of action of lincomycin and related antibiotics,** In Mitsuhashi, S. (ed.), Drug action and drug resistance in bacteria. Baltimore: University Park Press; 1971. p. 305-336.

52. Vazquez D. 1985. **Macrolides, lincosamides, and streptogramins-early observations on their modes of action**. J Antimicrob Chemother 16 (Suppl. A): 225-226.

53. Nissen P, Hansen J, Ban N, Moore PB, Steitz TA. 2000. **The structural basis of ribosome activity in peptide bond synthesis**. Science 289: 920–930.

54. Moore PB, Steitz TA. 2002. **The involvement of RNA in ribosome function**. Nature 418: 229–235.

55. Andersson S, Kurland CG. 1987. **Elongating ribosomes *in vivo* are refractory to erythromycin**. Biochim 69: 901–904.

56. Vazquez D. 1979. **Inhibitors of Protein Biosynthesis (Molecular Biology, Biochemistry and Biophysics**. J Basic Microbio 30: 483-484.

57. Weisblum B. 1995. **Erythromycin resistance by ribosome modification**. Antimicrob Agent Chemother 39: 577–585.

58. Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J. 1999. **Distribution of gene encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci**. Antimicrob Agent Chemother 43: 1062-1066.

59. Zolezzi PC, Calvo MC, Millan L, Goni P, Canales M, Capilla S, Duran E and Gomez-Lus R. 2004. **Macrolide resistance phenotypes of commensal viridans group streptococci and *Gemella* spp**. **and PCR detection of resistance genes.**International J Antimicrob Agent 23: 582-589.

60. Alcaide F, Catarrala J, Linares J, Gudiol F and Martin R. 1996. **In vitro activities of eight macrolide antibiotics and RP-59500 (quinupristin/dalfopristin) against viridans group streptococci isolated from blood of neutropenic cancer patients.** *Antimicrob Agent Chemother* 40: 2117–2120.

61. Akiyama K, Taniyasu N, Hirota J, Iba Y & Maisawa K. 2001. **Recurrent aortic valve endocarditis caused by *Gemella morbillorum*. Report of a case and review of the literature.** *Jpn. Circ. J.* 65: 997–1000.

62. Adam D & Scholz H. 1996. **Five days of erythromycin estolate versus ten days of penicillin V in the treatment of group A streptococcal tonsillopharyngitis in children: Pharyngitis Study Group**. Clin Infect Dis 15: 712–7.

63. Hooton TM. 1991. **A comparison of azithromycin and penicillin V for the treatment of streptococcal pharyngitis**. Am J Med 91: S23–6.

64. Stein GE, Christensen S & Mummaw N. 1991. **Comparative study of clarithromycin and penicillin V in the treatment of streptococcal pharyngitis.** Clin Infect Dis 10: 949–53.

65. Jensen MM, Wright DN & Robison RA. **Microbiology for the health sciences.** 4th edn. New Jersey: Simon and Schuster / A Viacom Company; 1997. 123-139.

66. Rezanka T, Spizek J & Sigler K. 2007. **Medicinal use of lincosamides and microbial resistance to them.** Anti-Infect Agents Med Chem 6: pp. 133–144.

67. Hoeksema H, Bannister B, Birkenmeyer RD, Kagan F, Magerlein BJ, MacKellar FA , Schroeder W, Slomp G & Herr RR. 1964. **Chemical studies of lincomycin**. The structure of lincomycin. J Am Chem Soc 86: pp. 4223–4224.

68. Jones WF, Nichols RL & Finland M. 1956. **Development of resistance and cross-resistance *in vitro* to erythromycin, carbomycin, spiramycin,**

oleandomycin and streptogramin. Proceed Soc Experim Biol Med 93: 388–93.

69. Leclercq R and Courvalin P. 1991. **Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification.** Antimicrob Agent Chemother 35: 1267-1272.

70. Leclercq R and Courvalin P. 1991. **Intrinsic and unusual resistance to macrolide, lincosamide, and streptogramin antibiotics in bacteria.** Antimicrob Agent Chemother 35: 1273-1276.

71. Leclercq R and Courvalin P. **Mechanisms of resistance to macrolides and functionally related antibiotics.** *In* Bryskier AJ, Butzler JP, Neu HC and Tulkens PM. (ed.), Macrolides-chemistry, pharmacology, and clinical uses. Paris: Arnette, Blackwell; 1993. p. 125-141.

72. Leclercq R. 2002. **Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications**. Clin Infect Dis 34: 482-92.

73. Rice L & Bonomo RA. **Genetic and biochemical mechanisms of bacterial resistance to antimicrobial agents.** In Antibiotics in Laboratory Medicine, 4th edn. Baltimore: Williams & Wilkins; 1996. pp. 453–51.

74. Sutcliffe J, Tait-Kamradt A & Wondrack L. 1996. ***Streptococcus pneumoniae*and *Streptococcus pyogenes* resistant to macrolides but**

sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob Agent Chemother 40: 1817–24.

75. Courvalin P, Ounissi H and Arthur M. 1985. **Multiplicity of macrolide lincosamide-streptogramin antibiotic resistance determinants**. J Antimicrob Chemother 16: 91–100.

76. Chung WO, Werckenthin C, Schwarz S and Roberts MC. 1999. **Host range of the *ermF* rRNA methylase gene in bacteria of human and animal origin**. J Antimicrob Chemother 43: 5–14.

77. Matsuoka M, Inoue M, Nakajima Y and Endo Y. 2002. **New *erm* Gene in *Staphylococcus aureus* Clinical Isolates**. Antimicrob Agent Chemother 46: p. 211–215.

78. Lai CJ, and Weisblum B. 1971. **Altered methylation of ribosomal RNA in an erythromycin-resistant strain of *Staphylococcus aureus.*** Proc. Natl Acad Sci USA 68: 856-860.

79. Fernandez-Munoz R, Monro RE, Torres-Pinedo R, and Vasquez D. 1971. **Substrate- and antibiotic-binding sites at the peptidyl-transferase centre of *Escherichia coli* ribosomes. Studies on the chloramphenicol, lincomycin and erythromycin sites.** Eur J Biochem 23: 185–193.

80. Bechhofer D. H. 1990. **Triple post-transcriptional control.** Mol Microbiol 4: 1419–1423.

81. Dubnau D. 1984. **Translational attenuation: the regulation of bacterial resistance to the macrolide-lincosamide-streptogramin B antibiotics**. Crit Rev Biochem 16: 103–132.

82. Weisblum B. 1985. **Inducible resistance to macrolides, lincosamides, and streptogramin type B antibiotics: the resistance phenotype, its biological diversity, and structural elements that regulate expression**. A rev J Antimicrob Chemother 16(Suppl. A): 63–90.

83. Weisblum B. 1995. **Insights into erythromycin action from studies of its activity as inducer of resistance**. Antimicrob Agent Chemother 39: 797–805.

84. Ross JI, Eady EA, Cove JH, Cunliffe WJ, Baumberg S and Wootton JC. 1990. **Inducible erythromycin resistance in staphylococci is encoded by a member of ATP-binding transport super-gene family**. Mol Microbiol 4: 1207–1214.

85. Lewis JS & Jorgensen JH. 2005**. Inducible clindamycin resistance in Staphylococci: should clinicians and microbiologists be concerned?** Clin Infect Dis 40: 280-5.

86. Levin TP, Suh B, Axelrod P, Truant AL & Fekete T. 2005. **Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: report of a clinical failure**. Antimicrob Agent Chemother 49: 1222-4.

87. Rao GG. 2000. **Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci?** J Antimicrob Chemother 45: 715.

88. Drinkovic D, Fuller ER, Shore KP, Holland DJ & Ellis-Pegler R. 2001. **Clindamycin treatment of Staphylococcus aureus expressing inducible clindamycin resistance**. J Antimicrob Chemother 48: 315-6.

89. Fasih N, Irfan S, Zafar A, Khan E & Hasan R. 2010. **Inducible clindamycin resistance due to expression of erm genes in Staphylococcus aureus: report from a tertiary care Hospital Karachi, Pakistan**. J Pak Med Assoc 60(9): 750-3.

90. HorinouchiS, ByeonWH & Weisblum B. 1983. **A complex attenuator regulates inducible resistance to macrolides, lincosamides, and streptogramin type B antibiotics in *streptococcus sanguis***. J Bacteriol 154: 1252-1262.

91. Eady EA, Ross JI, Tipper JL, Walters CE, Cove JH and Noble WC. 1993. **Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci**. J Antimicrob Chemother 31: 211–217.

92. Janosi L, Nakajima Y and Hashimoto H. 1990. **Characterization of plasmids that confer inducible resistance to 14-membered macrolides and streptogramin type B antibiotics in *Staphylococcus aureus***. Microbiol Immunol 34: 723–735.

93. Matsuoka M, Endou K, Saitoh S, Katoh M and Nakajima Y. 1995. **A mechanism of resistance to partial macrolide and streptogramin B antibiotics in *Staphylococcus aureus* clinically isolated in Hungary**. Biol Pharm Bull 18: 1482–1486.

94. Matsuoka M, Janosi L, Endou K, Saitoh S, Hashimoto H and Nakajima Y. 1993. **An increase of 63kDa-protein present in the cell membranes of Staphylococcus aureus that bears a plasmid mediating inducible resistance to partial macrolide and streptogramin B antibiotics**. Biol Pharm Bull 16: 1288–1290.

95. Milton ED, Hewitt CL and Harwood CR. 1992. **Cloning and sequencing of a plasmid-mediated erythromycin resistance determinant from *Staphylococcus xylosus***. FEMS Microbiol Lett 97: 141–147.

96. Ross JI, Eady EA, Cove JH and Baumberg S. 1995. **Identification of a chromosomally encoded ABC-transport system with which the staphylococcal erythromycin exporter MsrA may interact**. Gene 153: 93–98.

97. Ross JI, Farrell AM, Eady EA, Cove JH and Cunliffe WJ. 1989. **Characterization and molecular cloning of the novel macrolide-streptogramin B resistance determinant from *Staphylococcus epidermidis***. J Antimicrob Chemother 24: 851–862.

98. Wondrack L, Massa M, Yang BV and Sutcliffe J. 1996. **A clinical strain of *Staphylococcus aureus* inactivates and effluxes macrolides.** Antimicrob Agent Chemother 40: 992–998.

99. Higgins CF, Hyde SC, Mimmack MM, Gileadi U, Gill DR, and Gallagher MP. 1990. **Binding protein-dependent transport systems**. J Bioenerg Biomembr 22: 571–592.

100. Lewis K. 1994. **Multidrug resistance pumps in bacteria: variations on a theme.** Trends Biochem Sci 19: 119–123.

101. Nikaido H. 1994. **Prevention of drug access to bacterial targets: permeability barriers and active efflux**. Science 264: 382–388.

102. Clancy J, Petitpas J, Did-Hajj F, et al. 1996. **Molecular cloning and functional analysis of a novel macrolide-resistance determinant,*mefA*, from *Streptococcus pyogenes*.** Mol Microbiol 22: 867–879.

103. Tait-Kamradt A, Clancy J, Cronan M, et al. 1997. ***mefE* is necessary for the erythromycin-resistance M phenotype in *Streptococcus pneumoniae***. Antimicrob Agent Chemother 41: 2251–2255.

104. Zhong P and Shortridge VD. 2000. **The role of efflux in macrolide resistance.** Drug Resist Update 3: 325–9.

105. Del Grosso M, Iannelli F, Messina C, et al. 2002. **Macrolide efflux genes *mef*(*A*) and *mef*(*E*) are carried by different genetic elements in *Streptococcus pneumoniae*.** J Clin Microbiol 40: 774–778.

106. Brisson-Noe A, Delrieu P, Samain D and Courvalin P. 1988. **Inactivation of lincosamide antibiotics in *Staphylococcus***. J Biol Chem 263: 15880–15887.

107. Devriese LA. 1980. **Two new types of resistance to lincomycin in pathogenic staphylococci from animals**. Ann Microbiol (Paris) 131: 261–266.

108. Leclercq R, Brisson-Noe A, Duval J, and Courvalin P. 1987. **Phenotypic expression and genetic heterogeneity of lincosamide inactivation in *Staphylococcus* spp**. Antimicrob Agent Chemother 31: 1887–1891.

109. Le Goffic F, Capmau ML, Abbe J, Cerceau C, Dublanchet A and Duval J. 1977. **Plasmid-mediated pristinamycin resistance: PH1A, a pristinamycin 1A hydrolase**. Ann Inst Pasteur (Paris) 128: 471–474.

110. Le Goffic F, Capmau ML, Bonnet ML, Cerceau C, Soussy CJ, Dublanchet A and Duval J. 1977. **Plasmid-mediated pristinamycin resistance: PACIIA, a new enzyme which modifies pristinamycin IIA**. J Antibiot 30: 665–669.

111. Andremont A, Gerbaud G, and Courvalin P. 1986. **Plasmid-mediated high-level resistance to erythromycin in *Escherichia coli*.** Antimicrob Agent Chemother 29: 515–518.

112. Arthur M, Andremont A and Courvalin P. 1987. **Distribution of erythromycin esterase and rRNA methylase genes in members of the family *Enterobacteriaceae* highly resistant to erythromycin.** Antimicrob Agent Chemother 31: 404–409.

113. Arthur M, Autissier D and Courvalin P. 1986. **Analysis of the nucleotide sequence of the *ereB* gene encoding the erythromycin esterase type II.** Nucleic Acids Res 14: 4987–4999.

114. Barthe´le´my P, Autissier D, Gerbaud G and Courvalin P. 1984. **Enzymatic hydrolysis of erythromycin by a strain of *Escherichia coli*: a new mechanism of resistance.** J Antibiot 37: 1692–1696.

115. Kono M, O'Hara K and Ebisu T. 1992. **Purification and characterization of macrolide 29-phosphotransferase type II from a strain of *Escherichia coli* highly resistant to macrolide antibiotics**. FEMS Microbiol Lett 97: 89–94.

116. Noguchi N, Emura A, Matsuyama H, O'Hara K, Sasatsu M and Kono M. 1995. **Nucleotide sequence and characterization of erythromycin resistance determinant that encodes macrolide 29-phosphotransferase I in *Escherichia coli*.** Antimicrob Agent Chemother 39: 2359–2363.

117. O'Hara K. 1994. **Application of nuclear magnetic resonance spectrometry to measure the activity of bacterial macrolide esterase.** Microbios 79: 231–239.

118. O'Hara K, Kanda T, Ohmiya K, Ebisu T and Kono M. 1989. **Purification and characterization of macrolide 29-phosphotransferase from a strain of *Escherichia coli* that is highly resistant to erythromycin**. Antimicrob Agent Chemother 33: 1354–1357.

119. Ounissi H, and Courvalin P. 1985. **Nucleotide sequence of the gene *ereA* encoding the erythromycin esterase in *Escherichia coli*.** Gene 35: 271–278.

120. Schmitz FJ, Sadurski R, Kray A, Boos M, Geisel R, Kohrer K, Verhoef J and Fluit AC. 2000. **Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals**. J Antimicrob Chemother 45: 891-894.

121. Forbes BA, Sahm DF, Weissfeld AS. **Diagnistic Microbiology**.12th ed. China: MOSBY; 2007. 254-264.

122. Clinical and Laboratory Standard Institute. 2007. **Perfprmance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement**. Vol 27. No.1. M100-S17.

123. Fiebelkorn KR, Crawford SA, McElmeel ML and Jorgensen JH. 2003. **Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci.** J Clin Microbiol 41: 4740-4.

124. Sutcliffe J, Grebe T, Tait-Kamradt A and Wondrack A. 1996. **Detection of Erythromycin-Resistant Determinants by PCR.** Antimicrob Agent Chemother p. 2562–2566.

125. Leclercq R, Bauduret F and Soussy C. 1989. **Selection of constitutive mutants of gram-positive cocci inducible resistant to macrolides, lincosamides and streptogramins (MLS): comparison of the selective effects of the MLS**. Pathol Biol 37: 568–572.

126. Molstad et al. **Strama – a Swedish working model for containment of antibiotic resistance**. Eurosurveillance 2008; 46: 1-4.

127. Livermore DM, MacGowan AP.and Wale MCJ. 1998. **Surveillance of antimicrobial resistance.** British Med J 317: 614-615.

128. Yilmaz G, Aydin K, Iskender S, Caylan R and Koksal I. 2007. **Detection and prevalence of inducible clindamycin resistance in staphylococci**. J Med Microbiol 56: 342–345.

129. Schmitz FJ, Verhoef J and Fluit AC. 1999. **Prevalence of resistance ton MLS antibiotics in 20 European university hospitals participating in the European SENTRY surveillance programme. The Sentry Participants Group.** J Antimicrob Chemother 43: 783-792.

130. Stirnimann W, Droz S, Matter L and Bodmer T. 1997. **Phenotypes of resistance to macrolide and lincosamide antibiotics in *Staphylococcus* species.** Clin Microbiol Infect 3: 702-705.

131. Klietmann W, Focht J and Nosner K. 1997. **Retrospective resistance pattern of clinical isolates in vitro against imipenem and other antimicrobial agents between 1986 and 1989.** Drug Investigation 3: 270-277.

132. Brien TF. 1987. **Resistance of bacteria to antibacterial agents: report of Task Force 2.** Rev Infect Dis 9: Suppl. 3: S244-260.

133. Doern GV, jones RN, Pfaller MA, Kugler KC and Beach ML. 1999. **Bacterial pathogens isolated from patients with skin and soft tissue infections: frequency of occurrence and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 1997).** The SENTRY Study Group (North America). Diagn Microbiol Infect Dis 34: 65-72.

134. Felmingham D and Gruneberg RN. 1996. **A multicentre collaborative study of the antimicrobial susceptibility of community-acquired, lower respiratory tract pathogens 1992-1993: the Alxander Project. The Alexander Project Group.** J Antimicrob Chemother 38: Suppl. A, 1-57.

135. Chambers HF. 1997. **Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications.** Clin Microbiol 37: 1913-1920.

136. Polyzou A, Slavakis A, Pournaras S, Maniatis AN, Sofianou D and Tsakris A. 2001. **Predominance of a methicillin-resistant *Staphylococcus***

aureus **clone susceptible to erythromycin and several other non-β-lactam antibiotics in a Greek hospital**. J Antimicrob Chemother 48: 231-234.

137. Aracil, Minambres B, Oteo M, Rosa JM, Gomez-Garces J, Alos JI. 2002. **Susceptibility of strains of *Streptococcus agalactiae* to macrolides and lincosamides, phenotype patterns and resistance genes**. Clin Microbiol Infect 8: 745-748.

138. Seppälä H, Klaukka T, Vuopio-Varkila J, Muotiala A, Helenius H, Lager K, Huovinen P and the Finnish Study Group for Antimicrobial Resistance.1997. **The Effect of Changes in the Consumption of Macrolide Antibiotics on Erythromycin Resistance in Group A Streptococci in Finland.** N Engl J Med 337: 441-446.

139. Pal N, Sharma B, Sharma R and Vyas L. 2010. **Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India**. J Postgrad Med 56(3):182-185.

140. Alos JI, Aracil B, Oteo J, Torres C, Gomez-Garces JL, and the Spanish Group for the Study of Infection in the Primary Helth Care Setting. 2000. **High prevalence of erythromycin-resistant, clindamycin/miocamycin-susceptible (M phenotype) Streptococcus pyogenes: results of a Spanish multicenter study in 1998.** J Antimicrob Chemother 45: 605-609.

141. George, M, Eliopoulos, Section Editor 2002. **Mechnisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications**.Clin Infect Dis 34: 482-492.

142. Hsueh PR, Teng LJ, Lee LN, Ho SW, Yang PC, Luh KT. Nov 2001. **High Incidence of Erythromycin Resistance among Clinical Isolates of *Streptococcus agalactiae* in Taiwan**. Antimicrob Agent Chemother 45(11): 3205–3208.

143. Hamilton-Miller MT and Shah S. 2000. **Patterns of phenotypic resistance to the macrolide-lincosamide-ketolide-streptogramin group of antibiotics in staphylococci**. J Antimicrob Chemother 46: 941-949.

144. Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F and Etienne J. 1999. **Distribution of genes encoding resistance to macrolides. Lincosamides, and streptogramins among staphylococci**. Antimicrob Agent Chemother 43: 1062-1066.

145. Nicola FG, McDougal LK, Biddle JW and Tenover FC. 1998. **Characterization of erythromycin-resistant isolates of *Staphylococcus aureus* recovered in the United States from 1958 through 1969**. Antimicrob Agent Chemother 42: 3024-3027.

146. Hill CAST, Finn R and Denye V. 1973. **Depression of Cellular Immunity in Pregnancy due to a Serum Factor**. British Med J 3: 513-514.

147. Scheibl P, Zerbe H. 2000. **Effect of progesterone on the immune system in consideration of bovine placental retention.** Dtsch Tierarztl Wochenschr 107 (6): 221-227.

148. Huland H and Busch R. 1984. **Pyelonephritic scarring in 213 patients with upper and lower urinary tract infections: Long-term follow-up**. J Urol 132:936–9.

149. Najar MS, Saldanha CL and Banday KA. 2009. **Approach to urinary tract infections**. Indian J Nephrol 19(4): 129–139.

150. Davis MF, Peterson AE, Julian KG, Greene WH, Price LB, Nelson K, Whitener CJ and Silbergeld EK. 2013. **Household Risk Factors for Colonization with Multidrug-Resistant *Staphylococcus aureus* Isolates.** doi: 10.1371/journal.pone.0054733.

151. Brzychczy-Wloch M, Borszewska-Kornacka M, Gulczynska E, Wojkowska-Mach J, Sulik M, Grzebyk M, Luchter M, Heczko PB, Bulanda M. 2013. **Prevalence of antibiotic resistance in multi-drug resistant coagulase-negative staphylococci isolated from invasive infection in very low birth weight neonates in two Polish NICUs.** Ann Clin Microbiol Antimicrob 12: 41.

152. Adam HJ, Baxter MR, Davidson RJ, Rubinstein E, et al. 2013.**Comparison of pathogens and their antimicrobial resistance patterns in paediatric, adult and elderly patients in Canadian hospitals.** J Antimicrob Chemother 68 (suppl 1): i31-i37.

جامعة النجاح الوطنية

كلية الدراسات العليا

دراسة حول مقاومة بعض الأنواع البكتيرية للمضادات الحيوية من النوع (Macrolides) والمشابهة وظيفيا لها في منطقة نابلس

إعداد

نائله خالد أسعد صبّاح

إشراف

د. معتصم المصري

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين.

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الملخص

تم جمع 200 عزلة بكتيرية من النوع Staphylococci و52 النوع Streptococci من عدد من المراكز الصحية في محافظة نابلس خلال الفترة الممتدة من كانون ثاني من العام 2012 ولغاية شهر نيسان من العام 2013 م .شملت العزلات أنواع بكتيرية جمعت من عينات طبية مختلفة. تم استخدام طريقة agar dilution method لتحديد قيمة MIC للمضادات الحيوية من النوع اريثروميسين والنوع كلنداميسين لكل العزلات باستثناء العزلات من النوع *S. pneumoniae* و التي استخدام لفحصها طريقة Micro-broth dilution method. تم فحصت 47 عزلة مختلفة و مقاومة للمضاد الحيوي اريثروميسين باستخدام تقنية ال PCR بهدف الكشف عن الجينات المسؤولة عن المقاومة و المتمثلة بالجينات *ermA, ermB, ermC, msr, mef, ere* . استخدمت نتائج القيم لكل من MIC للضادات الحيوية المستخدمة و فحص التحفيز على مقاومة المضاد الحيوي كلنداميسين وكذلك نتائج الكشف عن الجينات ذات العلاقة بمقاومة المضادات لغرض تحديد آلية المقاومة للمضادات الحيوية لدى الأنواع البكتيرية قيد الدراسة. لوحظ وجود نسب مقاومة عالية للمضاد الحيوي اريثروميسين لدى العزلات البكتيريا من النوع Streptococci (63%) وكذلك في النوع Staphylococci (65.5%) في حين كانت نسبة المقاومة لهذا المضاد الحيوي في النوع Coagulase negative staphylococci (76.9%) أعلى مما هي عليه في النوع *S. aureus* (64.7%). أما بالنسبة للمضاد الحيوي كلنداميسين كانت نسب المقاومة 48.1% في البكتيريا Streptococci و20.5% في من النوع Staphylococci . وبينت الدراسة أن مقاومة المضاد الحيوي من

النوع اريثروميسين في البكتيريا من النوع Staphylococci اعتمدت على آلية الضخ (efflux mechanism) في % 50.4 من العزلات وعلى آلية تغير شكل هدف المضاد الحيوي في البكتيريا (Target modification) في 49.6%. وأما بالنسبة للبكتيريا Streptococci فاعتمدت على التغير في شكل الهدف في غالبية العزلات (75.8%) لهذا المضاد الحيوي. كما تبين أن % 24.2 من هذا النوع البكتيري اعتمد على آلية ضخ المضاد الحيوي.

أما بالنسبة لنتائج الكشف عن الجينات ذات العلاقة بمقاومة المضادات الحيوية باستخدام تقنية ال PCR في 36 عزلة من النوع Staphylococci لوحظ وجود ألجين *msr* و*ermC* و *ermA* بالنسب 55.6% و30.6% و25% على التوالي. أما في ما يتعلق بدور هذه الجينات في العزلات البكتيرية من نوع Streptococci فقد لوحظ وجود الجينات *ermB* ، *mef*، *ere* ، *ermC* موزعة على التوالي في 11 عزلة 81.8، 27.3، 9.1 و 9.1% .

أما في ما يتعلق بمقاومة المضاد الحيوي اريثروميسين في البكتيريا من النوع Staphylococci فقد كانت أعلى عند الأطفال الرضع في الفئة العمرية 0–2 سنة (74.5%) والأشخاص كبار السن (75%) وبالمثل كانت المقاومة للمضاد الحيوي كلنداميسين في البكتيريا من النوع Staphylococci مرتفعة عند الأشخاص كبار السن (50%) وكانت هذه النسبة ذات دلالة إحصائية (***P***=0.000) بالمقارنة بالفئة العمرية 3–14 سنة.

لقد كانت مقاومة البكتيريا Staphylococci للمضاد الحيوي اريثروميسين في قسم الأمراض النسائية الأعلى بالمقارنة مع باقي عزلات الأقسام الأخرى و كانت لهذه الفروقات دلالة إحصائية (***P***=0.000). كما لوحظ ارتفاع في نسبة المقاومة للمضاد الحيوي اريثروميسين في البكتيريا المعزولة من عينات الدم و من التجويف الأنفي بنسب ذات دلالة إحصائية بالمقارنة مع تلك المعزولة من مسحات الجروح. أن وجود ما نسبة 19.1% من العزلات البكتيرية من النوع Staphylococci المقاومة للمضاد الحيوي اريثروميسين و التي اعتمدت آلية الحث (Inducible MLS_B phenotype) في المقاومة لهي ظاهرة تستدعي انتباه الأطباء و المعنيين في اتخاذ سياسات استخدام هذه المضادات حيث أن الكشف عن المقاومة للمضاد

الحيوي كلنداميسين في هذه العزلات يحتاج إلى استخدام الفحص ألحثي (Induction test) لأن الطريقة التقليدية Disk diffusion وكذلك فحص ال MIC لا يمكنها الكشف عن نمط هذه المقاومة (Inducible mode). وأن العزلات ذات نمط التحفيز للمقاومة (Inducible MLS_B Phenotype) تمتلك الجين *erm* الذي يمكن أن يحول العزلة الحساسة للمضاد الحيوي كلنداميسين إلى عزلة مقاومة أثناء العلاج باستخدام هذا المضاد.